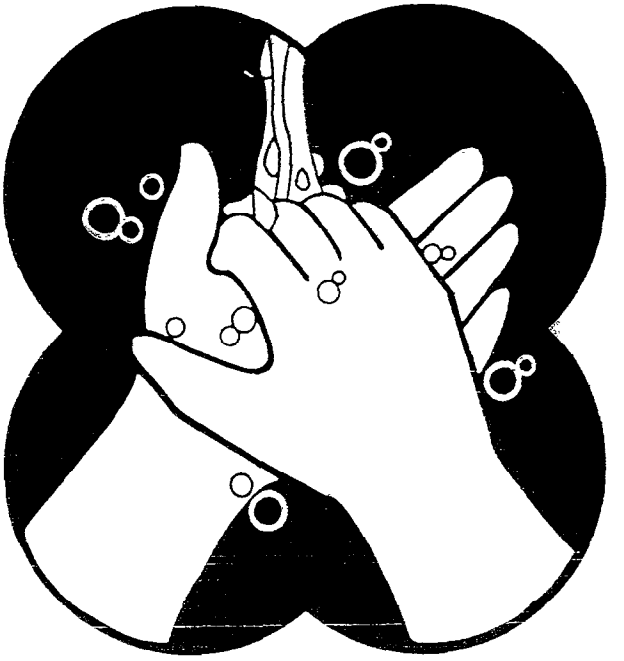


Bacti-Stat®

3000 PPM (0.3%) Triclosan
Health Care Personnel
Handwash

Research Bulletin

ECOLAB®

CONTENTS

Bacti-Stat® CHG AND CHLORHEXIDINE GLUCONATE

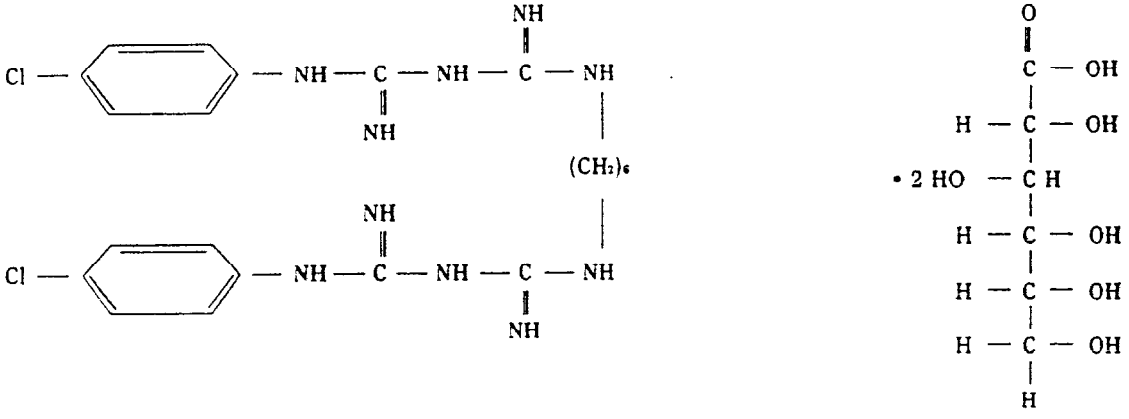
Bacti-Stat CHG Antimicrobial Solution with 2% Chlorhexidine Gluconate (CHG) also containing 4% Isopropyl Alcohol is recommended for use as a Health Care Personnel Handwash. These recommendations are based on **Bacti-Stat CHG** broad spectrum of activity against gram-positive and gram-negative bacteria, fungi (both yeasts and molds) and viruses.

Bacti-Stat CHG is effective in reducing the number of resident and transient bacteria commonly found in a clinical setting. This reduction is both immediate and persistent. **Bacti-Stat CHG** has been tested and proven effective against a broad spectrum of gram-positive and gram-negative bacteria associated with nosocomial infections. The test results listed in this bulletin substantiate the activity of **Bacti-Stat CHG**.

Bacti-Stat CHG is an antimicrobial solution designed to diminish dermal flora. **Bacti-Stat CHG** contains surfactants (detergents) for cleansing the skin, emollients to help the skin maintain its natural oils and moisture level, and chlorhexidine gluconate to provide both bactericidal action and bacteriostatic properties against a wide range of microorganisms.

CHEMISTRY

Bacti-Stat CHG contains the antimicrobial agent Chlorhexidine Gluconate (CHG) also known as 1,1'-hexamethylenebis [5 (4-chlorophenyl) biguanide] digluconate.
The chemical structure of this compound is



The mode of action for CHG has been shown to differ at high and low concentrations. Concentrations below 100 ppm have bacteriostatic properties which act on cell membrane components. Concentrations above this level have bactericidal effects due to rapid action on the cytoplasm.

Bacti-Stat® CHG IS EFFECTIVE AS A HEALTH CARE PERSONNEL HANDWASH

In a clinical study to substantiate the effectiveness **Bacti-Stat CHG** as a Health Care Personnel Handwash, tests were conducted to mimic the use of a handwash in a clinical setting. A glove juice technique was employed to determine the percent reduction of the transient bacteria on the subjects' hands and forearms.

A baseline bacterial count was determined for each subject, by contaminating the hands and forearms with *Serratia marcescens* and washing with a buffered non-antimicrobial solution.

After the baseline determinations were completed, each subject repeated the *Serratia marcescens* contamination procedure. The subjects then washed for 30 seconds with **Bacti-Stat CHG**. This procedure was repeated 25 times. Glove juice samples were collected after the 5th, 10th, 15th, 20th, and 25th washings.

TEST DATA:

Percent Reduction of Transient Bacteria as determined by a Glove Juice Test using **Bacti-Stat CHG**

Wash Number	Bacterial Count
0 (base line before first wash)	1.85×10^4 *Serratia marcescens*
	% Bacterial Reduction
5th wash	99.99
10th wash	99.99
15th wash	99.99
20th wash	99.99
25th wash	99.99

Test data indicates that **Bacti-Stat CHG** delivers greater than a 99.9% reduction in the transient bacteria count when used as a Health Care Personnel Handwash. No adverse effects from repeated use were observed.

CONCLUSION

Bacti-Stat CHG effectively reduces the transient bacterial count needed (greater than 99.9%) without adverse effects to the skin. These properties built into **Bacti-Stat CHG** make it an ideal Health Care Personnel Handwash.

Bacti-Stat® CHG ACTIVE AGAINST GRAM-POSITIVE BACTERIA

MINIMUM INHIBITORY CONCENTRATION (a measure of bacteriostatic activity)

To substantiate the effectiveness of **Bacti-Stat CHG** against gram-positive bacteria, tests were conducted to determine the Minimum Inhibitory Concentration (MIC) of **Bacti-Stat CHG** *in vitro* against representative pathogenic gram-positive bacteria.

TEST:

Dilutions of **Bacti-Stat CHG** were made with Trypticase Soy Broth to obtain final CHG concentrations ranging from 100 ppm to 0.5 ppm The final culture suspension in the broth/**Bacti-Stat CHG** tubes had densities of between 10^5-10^6 CFU/ml. The tube containing the lowest concentration of **Bacti-Stat CHG** in which no turbidity was observed after 48 hours of incubation is the Minimum Inhibitory Concentration (MIC). **Bacti-Stat CHG** contains 20,000 ppm CHG.

RESULTS:

Microorganism	Strain	MIC of Bacti-Stat CHG (ppm CHG)
Bacillus cereus	ATCC 11778	2.5
Bacillus subtilis	ATCC 19659	1.0
Corynebacterium xerosis	ATCC 373	0.5
Leuconostoc mesenteroides	ATCC 13146	1.0
Micrococcus luteus	ATCC 381	0 5
	ATCC 4698	0.5
	Clinical Isolate	4.0
Micrococcus roseus	ATCC 186	0.5
Staphylococcus aureus	ATCC 6538	2.5
	ATCC 33592	2.5
	Clinical Isolate	1.0
	ATCC 25923	4 0
Staphylococcus epidermidis	ATCC 12228	2.5
	ATCC 12228	4.0
	ATCC 14990	0.5
	Clinical Isolate	2.5
Streptococcus (group D)	ATCC 12389	1.0
Streptococcus sp.	ATCC 12388	0.5
Streptococcus faecalis	ATCC 19433	4.0
Streptococcus pneumoniae	ATCC 6303	2.5
Streptococcus pyogenes	ATCC 19615	0.5
	ATCC 19615	4.0

CONCLUSION

Bacti-Stat CHG effectively inhibits the growth of known pathogenic gram-positive bacteria.

KILL TIME STUDIES (a measure of bactericidal activity)

To demonstrate the bactericidal properties of **Bacti-Stat CHG** against gram-positive bacteria, Kill Time Studies were conducted against representative gram-positive bacteria commonly found in a clinical setting:

TEST:

Bacti-Stat CHG was inoculated with a 24 hour culture of each of the test organisms. An aliquot of each inoculated **Bacti-Stat CHG** sample was removed at 15 seconds, 30 seconds, 1, 3 and 6 minute intervals, placed into subculture tubes containing neutralizers and incubated for 48 hours. The time necessary to elicit a 4 $\log_{10}$ reduction in bacterial count is the stated Kill Time.

RESULTS:

Microorganism	Strain	Kill Time for Bacti-Stat CHG (seconds)
Bacillus cereus	ATCC 11778	15
Bacillus subtilis	ATCC 19659	15
Leuconostoc mesenteroides	ATCC 13146	15
Micrococcus luteus	ATCC 381	15
	ATCC 4698	15
Micrococcus roseus	ATCC 186	15
Staphylococcus aureus	ATCC 6538	60
	ATCC 33592	30
	Clinical Isolate	60
Staphylococcus epidermidis	ATCC 12228	15
	ATCC 14990	15
	Clinical Isolate	30
Streptococcus (Group D)	ATCC 12389	30
Streptococcus sp.	ATCC 12388	15
Streptococcus pneumoniae	ATCC 6303	15
Streptococcus pyogenes	ATCC 19615	15

The Kill Time for **Bacti-Stat CHG** against known pathogenic gram-positive bacteria ranged from 15-60 seconds.

CONCLUSION

Bacti-Stat CHG has quick bactericidal activity against a wide range of known pathogenic gram-positive bacteria.

Bacti-Stat CHG IS ACTIVE AGAINST GRAM-NEGATIVE BACTERIA

MINIMUM INHIBITORY CONCENTRATION (a measure of bacteriostatic activity)

To substantiate the effectiveness of **Bacti-Stat CHG** against gram-negative bacteria, tests were conducted to determine the Minimum Inhibitory Concentration (MIC) of Bacti-Stat CHG *in vitro* against representative pathogenic gram-negative bacteria.

TEST:

Dilutions of **Bacti-Stat CHG** were made with Trypticase Soy Broth to obtain final CHG concentrations ranging from 100 ppm to 0 5 ppm. The final culture suspension in the broth/**Bacti-Stat CHG** tubes had densities of between 10^5-10^6 CFU/ml. The tube containing the lowest concentration of CHG which no turbidity was observed after 48 hours of incubation is the Minimum Inhibitory Concentration (MIC). **Bacti-Stat CHG** contains 20,000 ppm CHG.

RESULTS:

Microorganism	Strain	MIC of Bacti-Stat CHG (ppm CHG)
Acinetobacter calcoaceticus	ATCC 19606	5.0
	ATCC 19606	8.0
Citrobacter freundii	ATCC 8090	5.0
	ATCC 8090	8.0
Enterobacter aerogenes	ATCC 13048	7.5
Enterobacter cloacae	ATCC 23355	5.0
	ATCC 23355	8.0
	Clinical Isolate	7.5
Escherichia coli	ATCC 25922	2.5
	ATCC 25922	8.0
	Clinical Isolate	2.5
Klebsiella pneumoniae	ATCC 13883	5.0
	ATCC 13883	8.0
Neisseria meningitidis	Clinical Isolate	5.0
	ATCC 13077	1.0
Proteus mirabilis	ATCC 13090	1.0
Proteus vulgaris	ATCC 25933	75.0
Pseudomonas aeruginosa	ATCC 13315	5 0
	ATCC 9027	7.5
	ATCC 15442	20.0
	ATCC 15442	64.0
	ATCC 27853	10.0
	ATCC 27853	32.0
	Clinical Isolate	20.0
	Clinical Isolate	20.0
	Clinical Isolate	10.0
	Clinical Isolate	20.0
Pseudomonas cepacia	ATCC 25416	15.0
	Clinical Isolate	7.5

CONCLUSION

Bacti-Stat CHG effectively inhibits the growth of known pathogenic gram-negative bacteria.

KILL TIME STUDIES (a measure of bactericidal activity)

To demonstrate the bactericidal properties of **Bacti-Stat CHG** against gram-negative bacteria, Kill Time Studies were conducted against representative gram-negative bacteria commonly found in a clinical setting.

TEST:

Bacti-Stat CHG was inoculated with a 24 hour culture of each of the test organisms. An aliquot of each inoculated **Bacti-Stat CHG** sample was removed at 15 seconds, 30 seconds, 1, 3, and 6 minute intervals, placed into subculture tubes containing neutralizers and incubated for 48 hours. The time necessary to elicit a 4 $\log_{10}$ reduction in bacterial count is the stated Kill Time.

RESULTS:

Microorganism	Strain	Kill Time for Bacti-Stat CHG (seconds)
Acinetobacter calcoaceticus	ATCC 19606	15
Citrobacter freundii	ATCC 8090	15
Enterobacter aerogenes	ATCC 13048	15
Enterobacter cloacae	ATCC 23355	15
	Clinical Isolate	15
Escherichia coli	ATCC 25922	15
	Clinical Isolate	15
Klebsiella pneumoniae	ATCC 13883	15
	Clinical Isolate	15
Neisseria meningitidis	ATCC 13077	15
	ATCC 13090	15
Proteus mirabilis	ATCC 25933	30
Proteus vulgaris	ATCC 13315	15
Pseudomonas aeruginosa	ATCC 9027	15
	ATCC 15442	15
	ATCC 27853	15
	Clinical Isolate	15
	Clinical Isolate	15
	Clinical Isolate	15
	Clinical Isolate	15
Pseudomonas cepacia	ATCC 25416	15
	ATCC 25609	15
	Clinical Isolate	15
Pseudomonas fluorescens	ATCC 13525	15
	Clinical Isolate	30
Salmonella choleraesuis	ATCC 10708	15
Salmonella typhi	ATCC 6539	15
Salmonella typhimurium	ATCC 14028	15
Serratia marcescens	ATCC 8100	15
	ATCC 29022	15
	Clinical Isolate	15
	Clinical Isolate	15
	Clinical Isolate	15
Shigella flexneri	ATCC 9199	15
Shigella sonnei	ATCC 12022	15
	ATCC 25931	15

The Kill Time for **Bacti-Stat CHG** against known pathogenic gram-negative bacteria ranges from 15-30 seconds.

CONCLUSION

Bacti-Stat CHG has quick bactericidal activity against known pathogenic gram-negative bacteria.

Bacti-Stat CHG IS ACTIVE AGAINST MICROBES PREVIOUSLY REPORTED AS CONTAMINANTS OF CHG SOLUTIONS

MINIMUM INHIBITORY CONCENTRATION (a measure of bacteriostatic activity)

To substantiate the efffectiveness of **Bacti-Stat CHG** against microbes previously reported to be contaminants of CHG solutions, tests were conducted to determine the Minimum Inhibitory Concentration (MIC) of **Bacti-Stat CHG** *in vitro* against these microorganisms.

TEST:

Dilutions of **Bacti-Stat CHG** were made with Trypticase Soy Broth to obtain final CHG concentrations ranging from 100 ppm to 0.5 ppm. The final culture suspension in the broth/**Bacti-Stat CHG** tubes had densities of between 10^5-10^6 CFU/ml. The tube containing the lowest concentration of CHG in which no turbidity was observed after 48 hours of incubation is the Minimum Inhibitory Concentration (MIC). **Bacti-Stat CHG** contains 20,000 ppm CHG.

RESULTS:

Microorganism	Strain	MIC of Bacti-Stat CHG (ppm CHG)
Alcaligenes denitrificans ss. xylosoxidans	ATCC 27061	15.0
Proteus mirabilis	ATCC 25933	75.0
	ATCC 7002	15.0
	ATCC 12453	15.0
Proteus vulgaris	ATCC 6380	20.0
	ATCC 8427	50.0
	ATCC 13315	5.0
Pseudomonas aeruginosa	ATCC 9027	7.5
	ATCC 15442	20.0
	ATCC 27843	10.0
	Clinical Isolate	20.0
	Clinical Isolate	20.0
	Clinical Isolate	10 0
	Clinical Isolate	20.0
Pseudomonas cepacia	ATCC 25416	15.0
	Clinical Isolate	7.5
Pseudomonas picketti	ATCC 27511	40.0
Serratia marcescens	Clinical isolate	7.5

CONCLUSION

Bacti-Stat CHG effectively inhibits the growth of representative bacterial species previously demonstrated to be contaminants of CHG solutions.

KILL TIME STUDIES (a measure of bactericidal activity)

To demonstrate the bactericidal properties of **Bacti-Stat CHG** against bacteria previously shown to be contaminants of CHG products, Kill Time Studies were conducted against these organisms.

TEST:

Bacti-Stat CHG was inoculated with a 24 hour culture of each of the test organisms. An aliquot of each inoculated **Bacti-Stat CHG** sample was removed at 15 seconds, 30 seconds, 1, 3, and 6 minute intervals, placed into subculture tubes containing neutralizers and incubated for 48 hours. The time necessary to elicit a 4 $\log_{10}$ reduction in bacterial count is the stated Kill Time.

RESULTS:

Microorganism	Strain	Kill Time for Bacti-Stat CHG (seconds)
Alcaligenes denitrificans ss. xylosoxidans	ATCC 27061	15
Providencia stuartii	ATCC 33672	15
	ATCC 35031	15
Proteus mirabilis	ATCC 25933	30
	ATCC 7002	15
	ATCC 12453	15
Proteus vulgaris	ATCC 6380	15
	ATCC 8427	15
	ATCC 13315	15
Pseudomonas aeruginosa	ATCC 9027	15
	ATCC 15442	15
	ATCC 27843	15
	Clinical Isolate	15
	Clinical Isolate	15
	Clinical Isolate	15
	Clinical Isolate	15
Pseudomonas cepacia	ATCC 25416	15
	ATCC 25609	15
	Clinical Isolate	15
Pseudomonas picketti	ATCC 27511	15
Serratia marcescens	ATCC 8100	15
	ATCC 29022	15
	Clinical Isolate	15
	Clinical Isolate	15
	Clinical Isolate	15

The testing shows that bacterial species previously linked with the contamination of CHG products and solutions, have no inherant resistance to **Bacti-Stat CHG** with 2% CHG. Kill Times in all cases were 15 seconds or 30 seconds.

CONCLUSION

Bacti-Stat CHG has quick bactericidal activity against previously known pathogenic contaminants of CHG products.

Bacti-Stat® CHG IS ACTIVE AGAINST ANTIBIOTIC-RESISTANT BACTERIA

Many species and strains of bacteria have developed resistance to various antibiotics. To substantiate the bacteriostatic and bactericidal effectiveness of Bacti-Stat CHG against these antibiotic-resistant bacteria, several multiple resistant strains of bacteria were tested.

The specific antibiotic resistance of each bacterial strain are noted in the following chart, and this is followed by the specific testing.

		Antibiotic resistance of organism (x)														
ORGANISM	STRAIN	Amikacin	Ampicillin	Carbenicillin	Cefamadole	Cefazolin	Cefoxitin	Chloramphenicol	Clindamycin	Erythromycin	Gentamicin	Kanamycin	Methicillin	Penicillin	Tetracycline	Tobramycin
Staphylococcus aureus	ATCC 33592										X		X			
Staphylococcus aureus	Clinical Isolate					X			X	X	X		X	X	X	X
Staphylococcus epidermidis	Clinical Isolate								X	X	X	X	X	X	X	X
Escherichia coli	Clinical Isolate		X	X								X			X	
Klebsiella pneumoniae	Clinical Isolate		X	X				X							X	
Pseudomonas aeruginosa	Clinical Isolate	X	X	X	X	X	X	X			X				X	

MINIMUM INHIBITORY CONCENTRATION (a measure of bacteriostatic activity)

To substantiate the effectiveness of **Bacti-Stat CHG** against antibiotic resistant bacteria, tests were conducted to determine the Minimum Inhibitory Concentration (MIC) of Bacti-Stat CHG *in vitro* against representative pathogenic antibiotic resistant bacteria.

TEST:

Dilutions of **Bacti-Stat CHG** where made with Trypticase Soy Broth to obtain final CHG concentrations ranging from 100 ppm to 0.5 ppm. The final culture suspension in the broth/**Bacti-Stat CHG** tubes had densities of between 10^5-10^6 CFU/ml. The tube containing the lowest concentration of CHG in which no turbidity was observed after 48 hours of incubation is the Minimum Inhibitory Concentration (MIC). **Bacti-Stat CHG** contains 20,000 ppm CHG.

RESULTS:

Microorganism	Strain	MIC of Bacti-Stat CHG (ppm CHG)
Staphylococcus aureus	ATCC 33592	2.5
Staphylococcus aureus	Clinical Isolate	1.0
Staphylococcus epidermidis	Clinical Isolate	2.5
Escherichia coli	Clinical Isolate	2.5
Klebsiella pneumoniae	Clinical Isolate	5.0
Pseudomonas aeruginosa	Clinical Isolate	20.0

CONCLUSION

Bacti-Stat CHG effectively inhibits the growth of known pathogenic antibiotic resistant bacteria.

KILL TIME STUDIES (a measure of bactericidal activity)

To demonstrate the bactericidal properties of **Bacti-Stat CHG** against antibiotic resistant bacteria, Kill Time Studies were conducted against representative antibiotic-resistant bacteria commonly found in a clinical setting.

TEST:
Bacti-Stat CHG was inoculated with a 24-hour culture of each of the test organisms. An aliquot of each inoculated **Bacti-Stat CHG** sample was removed at 15 seconds, 30 seconds, 1, 3, and 6 minute intervals, placed into subculture tubes containing neutralizers and incubated for 48 hours. The time necessary to elicit a 4 $\log_{10}$ reduction in bacterial count is the stated Kill Time.

RESULTS:

Microorganism	Strain	Kill Time for Bacti-Stat CHG (seconds)
Staphylococcus aureus	ATCC 33592	30
Staphylococcus aureus	Clinical Isolate	60
Staphylococcus epidermidis	Clinical Isolate	30
Escherichia coli	Clinical Isolate	15
Klebsiella pneumoniae	Clinical Isolate	15
Pseudomonas aeruginosa	Clinical Isolate	15

The Kill Time for **Bacti-Stat CHG** against known pathogenic antibiotic resistant bacteria ranged from 15-60 seconds.

CONCLUSION

Bacti-Stat CHG has quick bactericidal activity against a wide range of known pathogenic antibiotic resistant bacteria.

Bacti-Stat CHG® IS ACTIVE AGAINST FUNGI (YEAST AND MOLDS)

MINIMUM INHIBITORY CONCENTRATION (a measure of fungistatic activity)

To substantiate the effectiveness of Bacti-Stat CHG against *Candida albicans*, a yeast, tests were conducted to show the Minimum Inhibitory Concentration (MIC) of Bacti-Stat CHG *in vitro* against this representative pathogenic fungus

TEST:

Dilutions of **Bacti-Stat CHG** were made with Trypticase Soy Broth to obtain final CHG concentrations ranging from 100 ppm to 0.5 ppm. The final culture suspension in the broth/**Bacti-Stat CHG** tubes had densities of between 10^5-10^6 CFU/ml. The tube containing the lowest concentration of CHG in which no turbidity was observed after 48 hours of incubation is the Minimum Inhibitory Concentration (MIC). **Bacti-Stat CHG** contains 20,000 ppm CHG.

RESULTS:

Microorganism	Strain	MIC of Bacti-Stat CHG (ppm CHG)
Candida albicans	ATCC 10231	5.0

CONCLUSION

Bacti-Stat CHG effectively inhibits the growth of this known pathogenic fungus.

KILL TIME STUDIES (a measure of fungicidal activity)

To demonstrate the fungicidal properties of **Bacti-Stat CHG** against known pathogenic fungi (both yeast and molds), Kill Time Studies were conducted against representative fungi commonly associated with clinical settings.

TESTS:

Test organisms were grown on Sabouraud-Dextrose agar for 5-7 days at 26-28°C. The mycelial mats were loosened from the agar surfaces, and the mycelial recoveries were macerated in PBS, vortex mixed, and filtered through sterile cotton gauze. The filtered conidial suspensions were quantitated, using the Petroff-Hausser Microscopic Counting Chamber Method, and the densitites standardized at a density of 5×10^6 conidia/ml.

5.0 ml aliquots of **Bacti-Stat CHG** were placed in test tubes. The tubes were placed in a water bath for 15 minutes, after which 0.2 ml of the fungal test organism was added.

Following the 15, 30, 45, or 60-second exposures to **Bacti-Stat CHG**, an APHA loopful of the mixture was transferred to glucose neopeptone agar plates, and incubated for 5-7 days at 26-28°C. The recovery agar, glucose neopeptone agar, contained 0.5% polysorbate-80 and 0.07% lecithin neutralizer. Two recovery plates were inoculated per time interval. Visible growth of the test organism on the recovery plates was confirmed to be the initial inoculum by preparing wet mounts of the surviving colonies and observing conidial and mycelial structures microscopically

RESULTS:

Organisms	Strain	Kill Time for Bacti-Stat CHG (seconds)
Aspergillus versicolor	ATCC 11730	15
Aspergillus niger	ATCC 6275	15
Blastomyces dermatitidis	ATCC 10225	15
Candida albicans	ATCC 10231	15
Microsporum audouinii	ATCC 10008	15
Microsporum canis	ATCC 36229	30
Microsporum gypseum	ATCC 14683	15
Mucor plumbeus	ATCC 6795	30
Penicillium notatum	CBS 15-6157	15
Penicillium roqueforti	CBS 15-6161	15
Pityosporum orbiculare	ATCC 44344	15
Pityosporum ovale	ATCC 14521	30
Rhizopus nigricans	ATCC 24862	15
Trichoderma viride	ATCC 32630	15
Trichophyton mentagrophytes	ATCC 18748	15
Trichophyton tonsurans	ATCC 28942	30

The Kill Time for **Bacti-Stat CHG** against various fungi ranged from 15-30 seconds.

CONCLUSION

Bacti-Stat CHG has quick fungicidal activity against known pathogenic fungi (both yeasts and molds).

Bacti-Stat CHG IS ACTIVE AGAINST VIRUSES

To substantiate the antiviral activity of **Bacti-Stat CHG** tests were conducted against the known pathogenic viruses Herpes simplex Type I (HSV I) and Herpes simplex Type II (HSVII).

TEST METHOD:

1. The test virus, HSV II, was propagated in Vero Cells. The viral infectivity titer of the HSV II test virus was determined to be $10^{6.0}$ $TCID_{50}$/ml. The test virus HSV I was propagated in Human Epithelial Cells. The viral infectivity titer of the HSV I test virus was determined to be $10^{5.5}$ $TCID_{50}$/ml.
2. 0.25 ml of the undiluted test viral suspensions was added to 0.25 ml of undiluted **Bacti-Stat CHG**. The test virus-**Bacti-Stat CHG** mixture was allowed to remain in contact for 15 seconds at room temperature (25-26°C). Treated and untreated (control) test viruses were titrated in Vero Cells, four monolayer roller tubes per dilution, and incubated at 36°C for five days. The treated and untreated titration monolayer tubes of cell cultures were observed daily during the incubation period, after which the end-point was calculated and recorded.
3. Viral recoveries were based on the observation for cytopathogenic effect (CPE) in the cell culture host systems.
4. Toxicity studies were performed to determine the non-specific toxicity of the **Bacti-Stat CHG** in the cell culture host systems. Observations of toxicity effects (CPE), were made following 24 hours of incubation at 36°C.
5. Test virus recoveries (viral viability versus viral inactivation) were demonstrated by cell culture host system inoculations and observing for CPE.
6. $TCID_{50}$, $TCLD_{50}$ and $TCTD_{50}$ calculations were effected, using the Reed-Munch Method.
7. Virus recovery confirmations were completed after exposure to A.O.A.C. phosphate buffer containing lecithin-Tween 80 neutralizer.
8. The untreated viral suspensions and the treated viral suspensions were titrated 10^{-1} through 10^{-8}.

RESULTS:

	Dilution of viral suspension HSV I	Dilution of viral suspension HSV II
Test Virus Control (Untreated) - TCID	$1.0 \times 10^{-5.5}$	1.0×10^{-6}
Test Virus & Bacti-Stat CHG - TCLD	1.0×10^{-2}	1.0×10^{-2}
CYTOTOXICITY Control (Bacti-Stat CHG) - TCTD	1.0×10^{-2}	1.0×10^{-2}

Herpes simplex Type I (HSV I)

$TCID_{50} = 1 \times 10^{-5.5}$

$TCLD_{50} = 1 \times 10^{-2}$

$TCTD_{50} = 1 \times 10^{-2}$

Viral Inactivation = Log Reduction of Virus Titer =

$TCID_{50} - TCLD_{50} = \underline{\underline{10^{3.5} \log_{10}}}$

Herpes simplex Type II (HSV II)

Reed-Munch $TCID_{50} = 1 \times 10^{-6}$

Reed-Munch $TCLD_{50} = 1 \times 10^{-2}$

Reed-Munch $TCTD_{50} = 1 \times 10^{-2}$

Viral Inactivation = Log Reduction of Virus Titer =

$TCID_{50} - TCLD_{50} = \underline{\underline{10^{4.0} \log_{10}}}$

Reed-Munch TCID—Tissue Culture Infectivity Dosage (median)
Reed-Munch TCLD—Tissue Culture Lethal Dosage (median)
Reed-Munch TCTD—Tissue Culture Toxicity Dosage (median)

CONCLUSION

Bacti-Stat CHG effectively inactivates known pathogenic viruses.

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ECOLAB

Huntington® brand

Cida-Rinse® Gel

What is it?

Cida-Rinse Gel is a waterless healthcare personnel antimicrobial hand rinse formulated with moisturizers to help condition and protect the skin.

Why use it?

Cida-Rinse Gel kills the germs that can lead to nosocomial infections. It dries quickly, without stickiness, and is ideal for situations when there is simply no time or place to wash.

How is it used?

Apply 1mL of *Cida-Rinse Gel* onto hands and rub until dry. Water is not required for rinsing. 540mL soap cartridges can be placed in wall-mounted dispensers or left freestanding on countertops. Place the 800mL bag-in-box refills directly into wall-mounted dispenser.

Where is it used?

Cida-Rinse Gel is designed for use in all settings where handwashing is not always possible. These include acute and sub-acute care facilities, long-term care facilities, clinics, beauty salons, dental offices, day care centers, laboratories, and animal health facilities.

When is it used?

Use *Cida-Rinse Gel* between contact with patients or the public and whenever you return to your workstation.

Product Specifications

Registration:
FDA OTC – Healthcare Personnel Hand Rinse
NDC – 47593-192-00

Microbiological:
Active ingredient: 70% ethyl alcohol antimicrobial agent proven effective against viruses, Gram-positive and Gram-negative bacteria, including HIV, Herpes, MRSA and *Pseudomonas aeruginosa*.

Color:
Blue

pH:
NA

Fragrance:
Fresh and clean

State:
Gel

Use temperature:
Room temperature

Prove it to yourself:
Apply *Cida-Rinse Gel* and rub onto hands. Note the cool, soft feel of moisturizers and the pleasant fragrance. Time the procedure — it's really quick.

Ecolab's Professional Products Division focuses on healthcare and hygiene management. Our sales force, the largest in the industry, provides consultation, troubleshooting and in-service training. We help you reduce chemical usage and labor costs, and improve sanitation/infection control.

FOR EMERGENCY MEDICAL INFORMATION CALL TOLL FREE 1-800-328-0026

SAFETY REMINDER: Before using this or any other product or dispensing system, make sure your employees read and understand the product label and Material Safety Data Sheet which contain appropriate hazard warnings and first aid instructions.

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ECOLAB

Huntington® brand

Cida-Rinse® Gel

Waterless Healthcare Personnel Antimicrobial Hand Rinse

Kills germs, soothes the skin

Key Questions

1. What qualities are most important to you in a waterless healthcare personnel hand rinse?
2. What areas of your facility could benefit most from a gel-based hand rinse?
3. What types of dispensing systems do you prefer?

Notes from call:

Feature	Benefit
70% ethyl alcohol active ingredient	Provides proven infection prevention. Effective against viruses and Gram-positive and Gram-negative bacteria, including MRSA, VRE and *Pseudomonas aeruginosa.*
Rapid antimicrobial action	Immediate protection – kills germs within 15 seconds. Lowers nosocomial infections.
One-step waterless procedure	No rinsing required. Saves on time, paper towel costs.
Convenient, economical gel formula	Eliminates wasteful dripping of liquid. Saves money and clean-up time.
Enriched with a balanced blend of moisturizers	Provides protection against irritation. Maintains skin's natural oils and moisture levels without stickiness. Promotes use.
[illegible] approved	Tested and proven effective as a healthcare personnel hand rinse.
	Promotes handwashing compliance.
Counter or wall-mounted disposable dispensers	Easy-to-use packaging saves time and money, minimizes contamination possibilities. Flexibility to meet any facility's needs.

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ENDURE™ 300 Cida-Rinse® Gel

What is it?

ENDURE 300 Cida-Rinse Gel is a waterless healthcare personnel antimicrobial hand rinse formulated with moisturizers to help condition and protect the skin.

Why use it?

ENDURE 300 Cida-Rinse Gel kills the germs that can lead to nosocomial infections. It dries quickly, without stickiness, and is ideal for situations when there is simply no time or place to wash.

How is it used?

Apply 1mL of *ENDURE 300 Cida-Rinse Gel* onto hands and rub until dry. Water is not required for rinsing. 540 mL soap cartridges can be placed in wall-mounted dispensers or left freestanding on countertops. Place the 800 mL bag-in-box refills directly into wall-mounted dispenser.

Where is it used?

ENDURE 300 Cida-Rinse Gel is designed for use in all settings where handwashing is not always possible. These include acute and sub-acute care facilities, long-term care facilities, clinics, beauty salons, dental offices, day care centers, laboratories, and animal health facilities.

When is it used?

Use *ENDURE 300 Cida-Rinse Gel* between contact with patients or the public and whenever you return to your workstation.

Product Specifications

Registration:
FDA OTC – Healthcare Personnel Hand Rinse
NDC – 47593-264-00

Microbiological:
Active ingredient: 70% ethyl alcohol antimicrobial agent proven effective against viruses, Gram-positive and Gram-negative bacteria, including HIV, Herpes, MRSA and *Pseudomonas aeruginosa*.

Color:
Blue

pH:
NA

Fragrance:
Fresh and clean

State:
Gel

Use temperature:
Room temperature

Prove it to yourself:
Apply *Endure 300 Cida-Rinse Gel* and rub onto hands. Note the cool, soft feel of moisturizers and the pleasant fragrance. Time the procedure — it's really quick.

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SAFETY REMINDER: Before using this or any other product or dispensing system, make sure your employees read and understand the product label and Material Safety Data Sheet – these contain appropriate hazard warnings and first aid instructions

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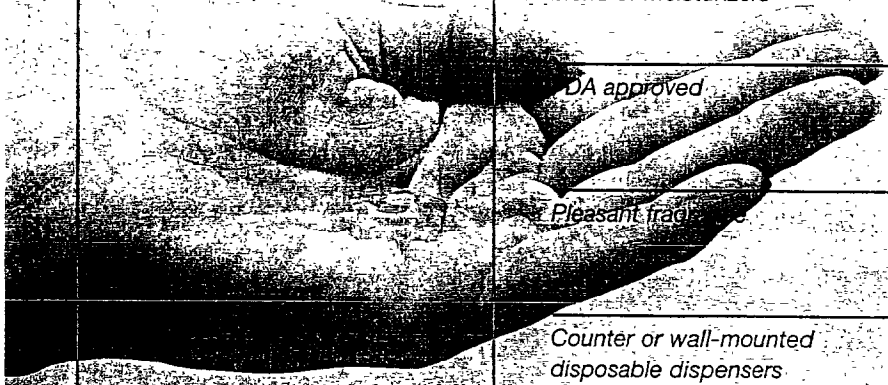
Key Questions

1. What qualities are most important to you in a waterless healthcare personnel hand rinse?
2. What areas of your facility could benefit most from a gel-based hand rinse?
3. What types of dispensing systems do you prefer?

Notes from call:



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Pleasant fragrance	Promotes handwashing compliance.
Counter or wall-mounted disposable dispensers	Easy-to-use packaging saves time and money, minimizes contamination possibilities. Flexibility to meet any facility's needs.

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Journal of Hospital Infection (1996) 32, 99–104

Contamination of central venous catheters. The skin insertion wound is a major source of contamination

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Summary: In a prospective controlled trial we compared the rates of catheter-tip contamination in central venous catheters inserted with or without skin contact. The study was designed so that each patient was their own control. All patients had a single-lumen central venous catheter and a Swan-Gantz sheet inserted through the skin. A Swan-Gantz catheter was inserted and retracted through the sheet thus avoiding contact with skin or subcutaneous tissue. Catheter-tip cultures were performed on removal of catheters. Thirty-three Swan-Gantz catheters were cultured and all were sterile. In the corresponding 33 sheets 16 (48·6%) yielded bacterial growth. Four of the sheets showed growth of more than 15 cfu. In the 26 single-lumen catheters, eight (30·8%) catheter-tips grew bacteria, and four of them had more than 15 colonies. The study supports the theory that the skin-insertion wound is a major source of catheter-contamination.

Keywords: Central venous catheters; catheter related sepsis; bacterial contamination.

Introduction

Contamination of central venous catheters is a common problem in the intensive care unit, and several studies have reported contamination or colonization rates of catheters to range between 3·8% and 4·7%.[1–6] Some studies have shown correlation between contamination of central venous catheters and septicaemia, and incidences of catheter-associated septicaemia between 2·5 and 25% have been documented.[7,1,5]

Many factors may contribute to the contamination of central venous catheters. Bjørnson *et al.* 1982[1], Maki *et al.* 1977[3], and Snydman *et al.* 1982*b*[6] suggest that most catheter-related infections begin as a local infection of the skin insertion wound. However, other studies implicate the catheter-administration set junction as an important source of bacterial contamination.[5,8,9] Recently, we have shown that the use of infusion-lines, even

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Table I. *Bacterial contamination of catheter-tips*

	Catheter type		
	Sheet	Swan-Gantz	Single-lumen
Sterile (No growth)	17 ($P<0.01$)	33 ($P<0.01$)	18
Broth enrichment (<5 colonies)	10	0	2
1–3 colonies	0	0	1
4–14 colonies	2	0	1
Contaminated (>15 colonies)	4	0	4
Total	33	33	26

Statistica: cross tabulation (no contamination/contamination). Sheet vs. single-lumen, not significant.

Table II. *Catheter-tip contamination in relation to time in situ and number of infusion-line breaks*

Catheter type	(*N*)	Time *in situ* (h)		Breaks (*N*)	
Sheet		Mean	(SEM)	Mean	(SEM)
No contamination	17	38·6	(4·6)	45 4	(5·7)
1–4 colonies	12	49·0	(5·6)	52·2	(6·5)
Contamination	4	44·4	(14·2)	62 3	(4·3)
Single-Lumen					
No contamination	18	57·9	(7·2)	5·7	(0·9)
1–4 colonies	4	61·9	(14·0)	6·2	(1·4)
Contamination	4	72·8	(0·9)	6·5	(1·0)
Swan-Gantz					
No contamination	33	30·6	(2·0)	1·8	(0·6)

The contamination rates for the catheters inserted with skin-contact (sheet and single-lumen), were significantly higher than the contamination rate for the Swan-Gantz catheters (no skin contact). There was no significant difference in contamination rates between sheets and single-lumen catheters. The statistical correlation in Table I is drawn between non-contaminated and significantly contaminated catheters.

Table II shows the results of catheter-tip cultures related to the time the catheters were *in situ*, and to the number of times the infusion-lines were broken during that time. No statistical correlation was found between time *in situ* and contamination, or between the number of breaks and contamination.

Staphylococcus epidermidis was isolated from six of eight contaminated catheters, and the remaining two (sheet and single-lumen catheter from

the same patient), yielded *Staphylococcus* and *Serratia* spp. *S. epidermidis* was the most frequently (75%) recovered from catheter-tips with low colony-counts and the less common species were *Klebsiella pneumoniae (2%)*, *Proteus* spp. and *Neisseria* spp. (1%).

Discussion

A correlation between contamination of central venous catheters and septicaemia in patients has been reported by several authors, and many attempts have been made to determine the major source of catheter contamination. A recent study,[10] performed by this group, concluded that even the heavy use of the infusion-lines, with multiple line breaks and infusions of blood and TPN, probably did not influence the contamination rates observed but indicated that the insertion-wound was a major source of contamination. This study supports the view that contamination-rates are not influenced by breaking the infusion-line. We found no significant difference between contamination-rates for sheets and for single-lumen catheters, and yet the sheet infusion-lines (Table II).

It is noteworthy that the Swan-Gantz catheters were all sterile. This suggests that the tips of the sheets were sterile when they were *in situ*, because retracting a Swan-Gantz catheter through a contaminated sheet-tip, would probably have resulted in at least some contamination. Thus, the sheet-tips must have been contaminated after retraction of the Swan-Gantz catheters, most likely when removing the sheet.

The nature of the organisms isolated, (many *S. epidermidis*), together with the fact that the sheets most likely became contaminated during retraction, strongly suggests that the skin insertion wound was the source of contamination. Bacteria present in the insertion wound can, in time, migrate along the catheter and into the bloodstream. In this study the catheters remained *in situ* for a relatively short period, which may explain the absence of catheter-related sepsis.

We conclude that the skin insertion wound is a major source of contamination of central venous catheters, and further studies on methods of reducing contamination would be of benefit.

We thank Dr Per Søgaard and Dr Jens Møller for their interest and cooperation, and for an excellent bacteriological service.

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Experiments on Antiviral Activity of Hand Disinfectants. Some Theoretical and Practical Considerations

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Received November 21, 1989 · Accepted November 30, 1989

Abstract

Various members of the picornavirus family, adenovirus 2, papovavirus SV40, as well as rotaviruses were tested for inactivation of infectivity by hand disinfectants formulated on the basis of ethanol. The inactivating effect of povidine-iodine (Betaisodona) on enteroviruses and rotaviruses was also investigated. The degree of inactivation by these disinfectants on the various non-enveloped viruses studied, however, was found unpredictable. In fact, striking differences with the very same disinfectant existed even for members of one virus family. Isopropanol was inactive against enteroviruses.

These findings are discussed from a theoretical point of view and practical implications are considered.

Disinfection of poliovirus 1-contaminated hands by 80% (v/v) ethanol proved not very effective, despite encouraging results obtained in the suspension test. The load of rotavirus-contaminated hands, however, was substantially reduced by treatment with Desderman. A practical regimen can be proposed.

Zusammenfassung

Verschiedene Mitglieder der Picornavirusfamilie, Adenovirus 2, Papovirus SV40 und Rotaviren wurden auf Inaktivierung ihrer Infektiosität durch Händedesinfektionsmittel auf der Basis von Ethanol untersucht. Der inaktivierende Effekt von Betaisodona auf Entero- und Rotaviren wurde auch bestimmt. Der Grad der Inaktivierung durch die untersuchten Desinfektionsmittel auf die verschiedenen nackten Viren erwies sich als unvorhersagbar. Selbst wenn dasselbe Desinfektionsmittel verwendet wurde, ergaben sich eklatante Unterschiede der Inaktivierung für Mitglieder *einer* Virusfamilie. Isopropanol war gegenüber Enteroviren inaktiv.

Die Befunde werden im Hinblick auf unsere Kenntnisse der Virusstruktur diskutiert, desgleichen werden praktische Vorschläge unterbreitet.

Die Desinfektion von Poliovirus-kontaminierten Händen durch 80% (v/v) Ethanol war wenig effektiv, obwohl im Suspensionstest ermutigende Resultate erzielt worden waren. Desderman reduzierte die nachweisbare Infektiosität Rotavirus-kontaminierter Hände erheblich unter für die Praxis geeigneten Bedingungen.

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Introduction

Inactivation of enveloped viruses presents little problems. A large number of lipid solvents and other agents destroys the infectivity of these viruses almost instantaneously (22). A different matter is the inactivation of non-enveloped viruses, and only limited information is so far available on this topic. On the other hand, nosocomial infections with, e.g., rota-, picorna- or adenoviruses are quite common and may lead to serious disease with sometimes lethal outcome (2, 6, 16, 17, 24, 26). It is also established that handwashing alone is often insufficient to free contaminated hands from virus (8, 23). In fact, on the basis of detailed experimental studies with polioviruses we have proposed the hypothesis that the tenacity of picornaviruses on skin may be the result of a special evolutionary process (23). At least the ease of horizontal spread of enteroviruses even under conditions of "high-standard hygiene" is more readily understood.

It is, therefore, of considerable medical importance to have available disinfectants with fast effects against non-enveloped viruses, which can also be applied to skin. Accordingly, we have tested alcohols and some alcoholic disinfectants on some representatives of non-enveloped virus groups in a suspension test. It seemed relevant for evaluation to follow the inactivation kinetics of virus infectivity, at least on a limited basis. Thus, unsuitable agents can be excluded. It appeared also desirable to supply further data whether test results obtained with one representative of a virus group would also apply to other members of the same group. Finally, 2 disinfectants found effective in the suspension test were used to disinfect poliovirus- or rotavirus-infected hands.

Material and Methods

Viruses

Poliovirus type 1 (Mahoney), type 2 (MEF_1), echovirus type 12 (Travis), and reovirus type 3 (Dearing) were described before (9), as well as echovirus type 11 (Porz) and papovavirus SV40 (777) (22). *D. Moldenhauer,* Erlangen, FRG, kindly supplied coxsackievirus type 4, strain Erlangen. Adenovirus type 2 (adenoid 6) was a gift of *R. Wigand,* Homburg, FRG. Vacciniavirus (Elstree) was obtained from *E. K. Kuwert,* Essen, FRG. *R. G. Wyatt,* Bethesda, MD, USA, kindly provided rotavirus strain Wa, *A. Delem,* Rixensart, Belgium, rotavirus, strain RIT 4237.

Cell cultures

GMK, HeLa, and CV-1 cells were described before (22). Ma 104 cells (25) were obtained from *R. G. Wyatt,* Bethesda, MD, USA.

J. Hilfenhaus, Marburg, FRG, kindly provided the Vero cells (29).

Virus propagation and infectivity assays

Enteroviruses were grown and titrated (plaque assay) in GMK cells (5). Rotaviruses and reovirus 3 were grown and assayed in Ma 104 cells (28). Rotavirus, strain Wa, and reovirus 3 were titrated by plaque assay (27), rotavirus, strain RIT 4237, by a quantal assay in tubes. Adenovirus 2 was propagated and assayed in HeLa cells, either by plaque assay (22) or by a quantal test in tubes. The propagation and plaque assay of SV40 in CV-1 cells has been

described before (22). Vacciniavirus was grown in Vero cells. Titration was done in tubes by a quantal test. The 50% endpoint of infectivity in all quantal tests was calculated by the method of *Reed* and *Muench* (19).

Disinfectants

The following substances were used.

1. Isopropanol, z.A., Merck, Darmstadt, FRG
2. Ethanol, absolute, "Baker Analyzed", J. T. Baker Chemicals B.V., Deventer, Holland
3. "V", Krueger GmbH, Bergisch Gladbach, FRG.
   Contents: ethanol, absolute 95,0 g
   glycerol 4.0 g
   ricinus oil 1.0 g
4. VP 1, J. Hoeffler, Hamburg, FRG.
   Contents: ethanol, 80% (w/w) 96.0 g
   choline dodecyl sulfonate 2.0 g
   citric acid 2.0 g
5. Desderman, Schülke u. Mayr, Norderstedt, FRG.
   Contents: ethanol, 95,3% (v/v) 78.2% (w/w)
   2,3,4,5-tetrabrome-6-methyl phenol 0.1% (w/w)
   excip. ad sol.
6. Betaisodona Lösung standardisiert, Mundipharma GmbH, Limburg, FRG.
   Contents: poly (1-vinyl-1-pyrrolidone)-iodine complex 10% (w/v).

Suspension test

Before the final proposal of the method recommended by the Bundesgesundheitsamt and the Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) (20) we used in the experiments with isopropanol and ethanol (Tables 1–6) a slight modification of the procedure, viz. 1 part of virus suspension was mixed with 14 parts disinfectant in a test tube. Later on (Tables 7–20), we followed exactly the procedure described by the Richtlinien (20), i.e. 1 part of virus suspension was mixed alternatively with 1 part of aqua bidestillata, with 1 part of fetal bovine serum (FBS), or with 1 part of 2% bovine serum albumin (BSA). Then 8 parts of disinfectant were added to the test tube, and the contents were rapidly mixed. This was considered time zero of the inactivation time. At indicated times aliquots of the mixture were drawn and diluted immediately into ice-cold phosphate-buffered saline (PBS) (4). A virus control was included in each experiment using distilled water instead of disinfectant.

The experiments were carried out in a water bath of 20±0.1 °C with reactants adapted to this temperature.

The concentrations of disinfectants indicated in the Tables refer to *initial* concentrations *before* mixing with virus and distilled water (FBS, or BSA). If no concentration is listed the disinfectant was used undiluted.

Rigorous controls for cytotoxic effects of the disinfectants were carried out (22). Tests for cytotoxicity in the tube tests were performed analogously to those described for the plaque test, i.e. besides evaluation of cell morphology in tubes inoculated with the corresponding concentrations of disinfectant also virus titrations were carried out in the presence of the relevant concentrations of disinfectant. Only concentrations of disinfectant not interfering with normal development of viral cytopathic effects were considered.

Hand tests

Thirty minutes prior to an experiment, a subject's hands were neither washed nor disinfected. Virus suspension (0.5 ml) was pipetted onto the subject's left palm and distributed with washing movements over the entire surface of both hands, wrists excluded. For this, 90 seconds were allowed, followed by a pause of 30 sec to let the suspension completey dry.

For recovery of virus from the hands, they were rinsed with washing and rubbing movements in 200 ml cold PBS in a glass Petri dish (diameter 20 cm) for 5 min.

The washing fluid and the virus suspension used for contamination of hands were titrated for infectivity and the proportion virus recovered was calculated.

For disinfection with ethanol (Table 21) the poliovirus-contaminated hands were rubbed twice for 90 sec with 2.5 ml each time. Subsequently, virus was recovered and titrated as just described.

Disinfection with Desderman of rotavirus-contaminated hands (Table 22) was carried out with 3 ml disinfectant for the time indicated. In case of the 3x1 min application of Desderman 3x3 ml were used. Virus recovery was performed as described above.

Results

Effects of various virus disinfectants in the suspension test

In a first series of experiments the inactivation effects on infectivity of several non-enveloped viruses by some disinfectants were tested in the suspension test. Though we are fully aware of the limitations of results obtained in a suspension test, particularly when compared with the efficacy of disinfectants on hands or other surfaces (7, 22) such studies may serve to exclude ineffective agents.

As shown in Table 1 isopropanol even in high concentrations has no effect on the infectivity of poliovirus 1 and echovirus types 11 and 12 after exposure for 15 min. This argues against the recommendation to use 70 per cent isopropanol for inactivation of picornaviruses (13) and agrees with results reported earlier (14).

Ethanol, on the other hand, in high concentrations, exhibits inactivating activity on poliovirus 1 and echovirus types 11 and 12 (Table 2). The effect of ethanol (80 and

Table 1. The infectivity of enteroviruses is not affected by various concentrations (v/v) of isopropanol within 15 min

	Virus control		Isopropanol, 15'				
	0'	15'	50%	60%	70%	80%	90%
Poliovirus 1	2.4×10^{7}*	2.0×10^{7}	1.8×10^{7}	1.1×10^{7}	1.4×10^{7}	1.6×10^{7}	2.4×10^{7}
Echovirus 11	2.8×10^{6}	2.4×10^{6}	2.4×10^{6}	1.7×10^{6}	2.0×10^{6}	1.8×10^{6}	2.4×10^{6}
Echovirus 12	4.5×10^{6}	4.2×10^{6}	4.8×10^{6}	5.0×10^{6}	4.1×10^{6}	3.4×10^{6}	3.7×10^{6}

* PFU

Table 2. Effect of various concentrations (v/v) of ethylalcohol on the infectivity of enteroviruses

	Virus control		Ethanol, 15'				
	0'	15'	50%	60%	70%	80%	90%
Poliovirus 1	1.5×10^{7}*	1.5×10^{7}	1.5×10^{7}	1.8×10^{7}	2.0×10^{6}	3.0×10^{2}	–
Echovirus 11	3.4×10^{6}	3.7×10^{6}	2.0×10^{6}	1.6×10^{5}	5.5×10^{3}	8.0×10^{2}	8.0×10^{1}
Echovirus 12	3.9×10^{6}	2.8×10^{6}	5.3×10^{5}	1.4×10^{5}	1.2×10^{2}	4.5×10^{1}	$<0.5\times10^{1}$

* PFU

Table 3. Inactivation of poliovirus 1 by ethanol (80% and 90% v/v) over time

	min					
	0	1	2	5	10	15
Virus control	2.9×10^{7}*					2.3×10^{7}
Ethanol, 80%		1.8×10^{6}	2.0×10^{4}	2.3×10^{3}	2.7×10^{2}	1.2×10^{2}
Ethanol, 90%		2.2×10^{2}	5.5×10^{1}	2.5×10^{1}	1.5×10^{1}	3.0×10^{1}

* PFU

Table 4. Effect of various concentrations (v/v) of ethanol on the infectivity of adenovirus 2 over time

	min				
	0	1	2	5	15
Virus control	6.2×10^{5}*				7.0×10^{5}
Ethanol, 60%		1.3×10^{5}	2.9×10^{4}	6.6×10^{2}	$< 0.5 \times 10^{1}$
Ethanol, 70%		2.4×10^{4}	4.4×10^{3}	3.5×10^{1}	$< 0.5 \times 10^{1}$
Ethanol, 80%		2.2×10^{4}	9.5×10^{2}	$< 0.5 \times 10^{1}$	$< 0.5 \times 10^{1}$
Ethanol, 90%		2.6×10^{4}	1.9×10^{3}	$< 0.5 \times 10^{1}$	$< 0.5 \times 10^{1}$

* PFU

Table 5. Effect of various concentrations (v/v) of ethanol on the infectivity of reovirus 3 over time

	min					
	0	1	2	5	10	15
Virus control	4.2×10^{5}*					11.5×10^{5}
Ethanol, 50%						19.5×10^{5}
Ethanol, 80%			4.7×10^{4}	3.0×10^{2}		
Ethanol, 90%		$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$

* PFU

Table 6. Inactivation of rotavirus (strain Wa) by various concentrations (v/v) of ethanol

Time (min)	Virus control	20%	40%	60%	90%
0	1.1×10^{5}				
1		1.0×10^{5}	7.8×10^{4}	$< 1.0 \times 10^{1}$	$< 1.0 \times 10^{1}$
2	1.1×10^{5}	1.1×10^{5}	8.4×10^{4}	$< 1.0 \times 10^{1}$	$< 1.0 \times 10^{1}$

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Table 8. I

* $TCID_5$

Table 9.

Virus co
"V" + A

* TCID

90%) on poliovirus 1 has been studied in some more detail (Table 3). It can be seen that 90% ethanol (v/v) reduces the infectivity of the virus about 10^5 fold within 1 min. Interestingly enough, adenovirus 2 appears to be slightly more resistant, though 80% ethanol reduces its infectivity below detectable levels (factor 10^5) by 5 min exposure time (Table 4). Reovirus 3 is rapidly inactivated by 90% ethanol (Table 5), rotavirus (strain Wa) even by 60% ethanol within 1 min (Table 6).

Another disinfectant with an ethanol component, disinfectant "V" has likewise been found effective against poliovirus 1; the presence of proteins like fetal bovine serum (FBS) (10% final concentration) or bovine serum albumin (BSA) (0.2% final concentration) did not interfere with the inactivating activity (Table 7). As expected, vaccinia virus is rapidly inactivated (Table 8), adenovirus 2 to a limited degree (Table 9). A representative of the papovavirus family, SV40, appears, unlike poliovirus, only slowly inactivated, and at most by a factor of 10^3 after 15 minutes (Table 10). The infectivity of rotavirus, on the other hand, is rapidly inactivated below detectable levels (factor almost 10^6) (Table 11).

Table 7. Inactivating effects of disinfectant "V" on the infectivity of poliovirus 1

	min				
	0	1	2	3	5
Virus control	$1.0 \times 10^{7*}$				1.0×10^7
"V" + A. bidest.		2.0×10^3	3.6×10^3	2.0×10^2	2.0×10^2
+ FBS		1.5×10^3	2.0×10^2	1.9×10^2	1.3×10^2
+ BSA		3.7×10^3	2.6×10^2	1.4×10^2	7.0×10^1

* PFU

Table 8. Inactivating effects of disinfectant "V" on the infectivity of vaccinia virus

	min	
	2	5
Virus control		$1.8 \times 10^{5*}$
"V" + A. bidest	$\leq 3.2 \times 30^1$	$\leq 3.2 \times 30^1$

* $TCID_{50}$

Table 9. Effects of disinfectant "V" on the infectivity of adenovirus 2

	min				
	0	1	2	3	5
Virus control	$3.2 \times 10^{4*}$				3.2×10^4
"V" + A. bidest.		0.5×10^3	2.0×10^3	2.0×10^2	6.0×10^2

* $TCID_{50}$

Table 10. Effects of disinfectant "V" on the infectivity of SV40

	min				
	0	1	2	3	5	15
Virus control	1.2×10^{7}*					8.5×10^{6}
"V" + A. bidest.		2.8×10^{5}	6.5×10^{4}	4.3×10^{4}	2.6×10^{4}	2.8×10^{4}
+ FBS		1.5×10^{5}	1.1×10^{5}	1.4×10^{5}	5.0×10^{4}	2.4×10^{4}
+ BSA		1.9×10^{5}	1.3×10^{5}	2.9×10^{4}	2.7×10^{4}	1.2×10^{4}

* PFU

Table 11. Inactivating effects of disinfectant "V" on the infectivity of rotavirus, strain RIT 4237

	min				
	0	1	2	3	5
Virus control	1.0×10^{6}*				1.8×10^{6}
"V" + A. bidest.		$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$
+ FBS		$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$
+ BSA		$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$

* $TCID_{50}$

A further alcoholic disinfectant, VP1, has also been investigated with members of non-enveloped virus families. As can be seen in Table 12, the infectivity of poliovirus 1 is inactivated almost instantaneously by the factor 10^5. The inactivating activity appears slightly impaired by the presence of FBS. Adenovirus 2 (Table 13) and SV40 (Table 14) are likewise inactivated within 1 minute below detectable levels, as is rotavirus (Table 15).

The virus-inactivating effects of the alcoholic disinfectant Desderman on a wide array of non-enveloped and enveloped viruses has been described previously (22). As shown in Table 16, Desderman also rapidly inactivates the infectivity of rotavirus, and the inactivation kinetics does not appear affected by the presence of proteins (data for BSA not shown). By lowering the concentration of Desderman by the factor of 0.625, the inactivating effect on rotavirus seems unimpaired. However, a further reduced concentration (factor 0.125) is practically ineffective (Table 16).

Table 12. Inactivation of poliovirus 1 by VP1

	min				
	0	1	2	3	5
Virus control	7.5×10^{6}*				6.5×10^{6}
VP1 + A. bidest.		$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$
+ FBS		1.7×10^{3}	1.0×10^{2}	0.5×10^{2}	0.5×10^{2}
+ BSA		$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$

* PFU

Table 13. Inactivating effects of VP1 on the infectivity of adenovirus 2

	min				
	0	1	2	3	5
Virus control	3.2×10^{4}*				1.8×10^{5}
VP1 + A. bidest.		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
+ FBS		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
+ BSA		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$

* $TCID_{50}$

Table 14. Inactivating effects of VP1 on the infectivity of SV40

	min				
	0	1	2	3	5
Virus control	1.2×10^{6}*				1.7×10^{6}
VP1 + A. bidest.		$< 0.5 \times 10^{2}$	$< 0.2 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$
+ FBS		$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$
+ BSA		$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$

* PFU

Table 15. Inactivating effects of VP1 on the infectivity of rotavirus, strain RIT 4237

	min				
	0	1	2	3	5
Virus control	5.6×10^{5}*				5.6×10^{5}
VP1 + A. bidest.		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
+ FBS		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
+ BSA		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$

* $TCID_{50}$

Table 16. Inactivating effects of Desderman on the infectivity of rotavirus, strain RIT 4237

		min			
		0	1	3	5
Virus control		1.8×10^{6}*			1.8×10^{6}
Desderman 100%	+ A. bidest.		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
	+ FBS		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
Desderman 62.5%	+ A. bidest.		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
	+ FBS		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
Desderman 12.5%	A. bidest.		1.8×10^{6}	5.6×10^{5}	5.6×10^{5}
	+ FBS		3.2×10^{6}	5.6×10^{5}	5.6×10^{5}

* $TCID_{50}$

The iodine-containing disinfectant Betaisodona was also tested against rotavirus. Concentrations as low as 0.1% inactivated the virus below detectable infectivity within 30 sec (Table 17). A tenfold lower concentration (0.01%) was ineffective. The inactivating effects of Betaisodona, however, appear significantly affected by the presence of proteins.

Table 17. Inactivation of rotavirus, strain RIT 4237, by Betaisodona

	min				
	0	1/2	1	2	5
Virus control	3.2×10^{6}*				5.6×10^{6}
Betaisodona 10%					
+ A. bidest.		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	
+ FBS		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
+ BSA		$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$	$\leq 3.2 \times 10^{1}$
Betaisodona 1.0%					
+ FBS				$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$
+ BSA				$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$
Betaisodona 0.1%					
+ A. bidest.		$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$	$\leq 3.2 \times 10^{0}$
+ FBS		5.6×10^{4}	5.6×10^{4}	1.8×10^{5}	5.6×10^{4}
+ BSA		1.8×10^{5}	1.8×10^{5}	1.8×10^{5}	1.8×10^{5}
Betaisodona 0.01%					
+ A. bidest.				1.8×10^{6}	5.6×10^{5}

* $TCID_{50}$

Differing disinfecting activity vs. various members of the same virus family

For obvious technical reasons only a very limited number of viruses can be selected to be tested against specified disinfectants (20). We have previously presented evidence that significant differences might occur with members of the same virus family, even with various strains of the same virus type (22). This finding has now been further substantiated.

It is obvious from Table 2 that echovirus types 11 and 12 are more sensitive to the disinfecting activity of 70% ethanol than poliovirus 1. Poliovirus 2, strain MEF_1 appears more sensitive to Betaisodona than poliovirus 1, strain Mahoney (Table 18), whereas echovirus 12, Travis, is rapidly inactivated below detectable level (factor $> 10^5$) (Table 18). A likewise striking example concerns the different sensitivities of poliovirus 1, strain Mahoney, and echovirus 11, strain Porz, respectively, versus disinfectant "V" (compare Tables 7 and 19).

Furthermore, echovirus types 11 (strain Porz) and 12 (prototype Travis) are significantly more resistent to disinfectant VP1 (Table 20) than poliovirus 1 (Mahoney) (see Table 12), particularly in the presence of proteins. A strain of coxsackievirus B4 (Erlangen), on the other hand, appears rapidly inactivated below detectable infectivity levels (Table 20).

Table 18.
virus 2, M

Poliovirus
Virus cont
Betaisodor
+ A. bi

Poliovirus
Virus cont
Betaisodor
+ A. bi

Echovirus
Virus cont
Betaisodor
+ A. bi

* PFU

Table 19.

Virus cont
"V" + A.
+ FB
+ BS

* PFU

Activity

Eighty
poliovirus
experimen
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Table 18. Different inactivating activity of Betaisodona on poliovirus 1, Mahoney, poliovirus 2, MEF_1, and echovirus 12, Travis

	min				
	0	10	20	30	40
Poliovirus 1					
Virus control	6.5×10^6*				6.0×10^6
Betaisodona 1.0%					
+ A. bidest.		4.5×10^5	2.0×10^2	$< 0.5 \times 10^2$	0.5×10^2
0.1%		6.5×10^5	6.5×10^4	8.5×10^1	1.5×10^1
Poliovirus 2					
Virus control	2.5×10^6				2.45×10^6
Betaisodona 1.0%					
+ A. bidest.		1.2×10^3	$< 0.5 \times 10^1$	$< 0.5 \times 10^1$	$< 0.5 \times 10^1$
0.1%		3.5×10^4	1.7×10^2	2.0×10^1	$< 0.5 \times 10^1$
Echovirus 12					
Virus control	1.5×10^6			1.2×10^6	
Betaisodona 1.0%					
+ A. bidest.		$< 0.5 \times 10^2$	$< 0.5 \times 10^2$	$< 0.5 \times 10^2$	
0.1%		$< 0.5 \times 10^1$	$< 0.5 \times 10^1$	$< 0.5 \times 10^1$	

* PFU

Table 19. Effects of disinfectant "V" on the infectivity of echovirus 11, strain Porz

	min				
	0	1	2	3	5
Virus control	2.8×10^7*				3.9×10^7
"V" + A. bidest.		2.4×10^7	2.4×10^5	1.7×10^4	3.2×10^3
+ FBS		2.7×10^7	2.1×10^5	8.5×10^3	3.7×10^3
+ BSA		2.7×10^7	2.3×10^5	1.4×10^4	1.4×10^3

* PFU

Activity of alcoholic disinfectants on virus-contaminated hands

Eighty % ethanol was shown in the suspension test to reduce the infectivity of poliovirus 1 within 2 min by the factor 10^3 (Table 3). In the light of our previous experiments (22, 23) and for practical purposes it seemed relevant to determine the disinfecting capacity of 80% ethanol with poliovirus 1-contaminated hands. After application of the virus (see Methods) the hands were rubbed twice carefully with 2.5 ml of the alcohol each time. For control the recoverability of virus from untreated hands had been determined before. The virus still to be recovered from the hands served as measure for the effectiveness of disinfection on hands. The results presented in Table 21 demonstrate that the recovery of poliovirus in the control is surprisingly reproducible from person to person. The effect of disinfection, however, was disappointing: as compared with the control the amount of infectious virus on hands was

Table 20. Different inactivating activity of VP1 on the infectivity of echovirus 11, Porz, echovirus 12, Travis, and coxsackievirus B4, Erlangen

	min				
	0	1	2	3	5
Echovirus 11					
Virus control	2.5×10^{7}*				9.5×10^{6}
VP1 + A. bidest.		5.5×10^{4}	1.5×10^{2}	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$
+ FBS		5.2×10^{6}	3.1×10^{6}	1.0×10^{6}	6.7×10^{5}
+ BSA		2.3×10^{6}	7.5×10^{4}	1.0×10^{4}	1.3×10^{3}
Echovirus 12					
Virus control	3.7×10^{6}				2.6×10^{6}
VP1 + A. bidest.		2.5×10^{4}	1.8×10^{4}	2.6×10^{3}	1.2×10^{2}
+ FBS		7.0×10^{5}	3.0×10^{5}	2.2×10^{5}	8.0×10^{4}
+ BSA		3.2×10^{5}	1.2×10^{5}	7.0×10^{4}	1.2×10^{4}
Coxsackievirus B4					
Virus control	3.1×10^{5}				2.4×10^{5}
VP1 + A. bidest.		0.5×10^{2}	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$
+ FBS		2.5×10^{2}	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$
+ BSA		0.5×10^{2}	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$	$< 0.5 \times 10^{2}$

* PFU

Table 21. Recovery of poliovirus 1, Mahoney, from untreated hands and from hands disinfected with 80% (v/v) ethanol

Poliovirus applied	Subject No.	Control	Ethanol	Percent recovered ethanol/control
8.75×10^{7}*				
	1	3.0×10^{7} (34%)**	4.5×10^{6} (5.1%)	14.9%
	2	2.8×10^{7} (32%)	7.5×10^{6} (8.6%)	26.8%
	3	2.9×10^{7} (33%)	1.5×10^{7} (17.1%)	52.5%

* PFU

** Percent recovered

Poliovirus-contaminated hands were rubbed 2× for 90 sec with 2.5 ml ethanol each time

reduced at maximum 7 fold, in one case only 2 fold (Table 21). Therefore effective disinfection on hands as compared to the results on the suspension test has been described before (22). *Nehrkorn* and *Steinmann*, however, reported more favorable results with 80% ethanol (18). This may be due to certain differences in experimental design. But we should like to stress that in our judgement slight experimental changes might change the tip of the balance, i.e., 80% ethanol does not appear a safe hand disinfectant vs. poliovirus.

Undiluted Desderman was demonstrated to inactivate rotavirus in the suspension test by the factor of about 10^{5} within 1 min (Table 16). Since disinfection of rotavirus-

contaminated hands is of considerable practical importance (1, 15), we tested the efficacy of Desderman.

In preliminary tests it was shown that about 20% of rotavirus strain RIT 4237 could be recovered from contaminated hands after rubbing about 10^6 to 10^7 infectious units into both hands (see Methods).

For disinfection virus-contaminated hands were rubbed with 3 ml of undiluted Desderman for various lengths of time: 5 min, 3 min and 1 min, respectively. The results are presented in Table 22. A 5 min treatment reduced recoverable infectivity on the average about 350 times as compared to the control. The best effect amounted to 0.014% or less, the least to 0.83% (factor about 120). The average efficacy of a 3 min treatment appears somewhat less (factor about 170), though the variability of the results must be considered.

A 1 min treatment (Table 22) could also be quite effective, but in one out of the 4 subjects tested with this regimen the result was poor (18.2%). A 3x1 min treatment did not seem strikingly better than a 3 min treatment (Table 22), though in the first situation each time 3 ml disinfectant had been used: thus the total amount of disinfectant was 3 times greater than in the standard test.

Table 22. Efficacy of disinfection under various conditions with Desderman of hands contaminated with rotavirus, strain RIT 4237

	Duration of disinfection			
	5 min	3 min	3 × 1 min	1 min
	percent			
	≤0.014*	0.041	0.14	≤0.012
	≤0.026	0.14	0.15	≤0.019
	0.028	0.36	0.20	2.3
	0.30	0.36	0.27	18.2
	0.33	0.76	0.68	
	0.51	1.90	1.58	
	0.83			
Average	≤0.291	0.59	0.50	≤5.13

* Percent recovered Desderman/control. Each figure refers to a different subject
Note: about 10^6 to 10^7 infectious units were applied to the hands. The average recovery of virus in the control was 20.1%

Discussion

As stated in the introduction relatively little is known on inactivation and inactivation kinetics of non-enveloped viruses by disinfectants. At least in part this is due to the difficult and costly methodological problems. Pioneering work with brilliant theoretical considerations has been done by *Sven Gard* and his group on the occasion of the development of the inactivated poliovirus vaccine (10). This, however, concerned inactivation of poliovirus infectivity by formaldehyde, a substance for various reasons unsuitable for application to skin.

J. M. Gwaltney and coworkers did important work on the spread of rhinoviruses by skin to skin contact and its prevention by virucidal compounds (3, 11, 12). In recent years *Sattar's* group studied the survival of rotaviruses on hands and its transfer to skin and stainless steel surfaces (1). Their results indicate the potential vehicular role for human hands in the spread of rotaviral infections, as shown previously in many studies on epidemiological grounds (see, e.g., 6, 15). However, thorough work on the effectiveness of disinfection of rotavirus-contaminated hands was still lacking. The importance of nosocomial and sometimes lethal picornavirus infections must also be stressed (16, 17, 24).

Work done over the last decade in our laboratory and by several others strongly emphasized the need for carefully controlled and highly standardized experimental conditions, actually a truism in experimental medicine. However, standardization for testing virucidal agents at least in the suspension test (20) on an international level materializes only slowly. The present work was done to assemble essential data on some alcoholic and one iodine-containing disinfectant to provide a basis for a critical discussion of standardized suspension tests. We further extended our work on disinfection of poliovirus- or rotavirus-contaminated hands.

Isopropanol, even in concentrations as high as 90%, had no inactivating effect on 3 different enteroviruses and, therefore, cannot be recommended for prevention of nosocomial infections of this virus group, as still done occasionally (see, e.g., 13). On the other hand, ethanol in high concentrations, e.g. 90%, inactivates the infectivity of poliovirus 1, reovirus 3 and rotavirus rather fast (Tables 3,5,6). Adenovirus 2 appears relatively resistant (Table 4). Adenovirus 2, a naked virus, was reported by *Klein* and *Deforest* (14) to be inactivated in a suspension test similar to enveloped viruses. The present data and previous results with adenovirus 5 and Desderman (22) do not support this finding.

Another alcoholic disinfectant tested in the present series, viz. "V" containing glycerol, appeared likewise not very effective against adenovirus 2 (Table 9), though considerably potent against poliovirus 1 (Table 7). Lastly, disinfectant VP1 (containing the tensid choline dodecyl sulfonate and citric acid) inactivated adenovirus 2 within 1 min below detectable infectivity (Table 13).

The papovavirus SV40 was readily inactivated by VP1 (Table 14), however considerably resistant to "V" (Table 10) and, as reported before, to Desderman (22).

Ready inactivation of rotavirus infectivity was achieved by all disinfectants tested in this series, by 60% ethanol (Table 6), by "V" (Table 11), VP1 (Table 15), Desderman (Table 16), and Betaisodona (Table 17), a finding of practical importance (see also 21).

Our understanding of the physicochemical basis of virion integrity is still much too incomplete to allow predictions as to the effects of certain groups of virucidal chemicals on viral infectivity. As demonstrated in this paper various disinfectants with an ethanol base affect the infectivity of a single virus, e.g. adenovirus 2 or SV40, quite differently, depending on additional components. Furthermore, the very same virucidal agent affected the representatives of various groups of naked viruses differently, and as yet unpredictively.

It appears that in regard to susceptibility to disinfectants the structural diversity of nonenveloped viruses is all in all greater than that of organisms such as bacteria, though we are aware of the limitations of such a broad statement. Enveloped viruses the infectivity of which is readily inactivated by destruction of the lipid-containing membrane, react almost uniformly, as one would expect.

A problem of considerable concern is the variability of various members of a single

virus family as to the susceptibility to the very same disinfecting agent. Striking examples were presented in the result section. Analogous to the situation just discussed for members of various virus groups predictions appear not possible. E.g., 70% ethanol inactivates echovirus types 11 and 12 significantly, but hardly poliovirus 1. On the other hand, the inactivating effects of VP1 vs. poliovirus 1 are in the order of 10^5, and also coxsackievirus B4 is rapidly inactivated, whereas the echoviruses tested are significantly resistant, particularly in the presence of fetal bovine serum. Furthermore, 0.1% Betaisodona hardly inactivates poliovirus 1, Mahoney, within 10 min (Table 18), but the infectivity of echovirus 12, prototype Travis, is reduced below detectable levels (factor $> 10^5$) within the same period of time (Table 18). It follows that a rational pattern is not yet in sight.

The practical implication concerns the selection of representative test viruses. It is obviously impossible to test larger numbers of virus types of one group, and possibly even various strains of one virus types. Whatever the practical solution will be, the present and previous studies (22) made it clear that a "universal" virucidal agent for skin disinfection will hardly be possible in the near future. Selective use of agents active against pathogenic viruses commonly present in certain settings must be the practical compromise.

Following a standard protocol for the suspension test is of utmost importance. We have earlier presented data on the significance of parameters like virus-disinfectant ratio, reaction temperature, and serum protein load (22). Variation of one of these parameters alone may alter the results dramatically, ranging from no effect on virus infectivity to inactivation over 4 orders of magnitude. We have substantiated these results in the present investigation (data not shown). However, it turned out informative to use at least 2 proteins to study the effect of serum protein load. Striking differences may become apparent (see e.g. Table 20).

Determination of inactivation kinetics over a certain time seems a necessity. A "curved" inactivation kinetics with a faster initial, and slower ensuing phase, often finally leveling off, appears almost a rule. The physicochemical problems involved were discussed by *Gard* (10). Therefore, depiciting only the time required for inactivation over a certain order of magnitude, e.g. reduction of infectivity by the factor of 100 or 1000, may curtail important information.

Criterium of a useful disinfectant will be its efficacy on hands. The methodological problems have been discussed in great detail before (22, 23). Suffice it to say here that the amount of infectious virus particles still on hands after washing or disinfection cannot be determined directly. This would require sampling periods in the order of hours. Furthermore, since poliovirus 1, and possibly many other viruses, stick to skin with greatly differing strength of binding after washing, the same effort of sampling may yield different fractions of the virus still on the skin, fractions the size of which is not known.

For practical reasons we have chosen a 5 min sampling period. In the control experiment about 4.2% of poliovirus 1, and about 20% of rotavirus, originally applied to hands could be recovered. The recovery rate was surprisingly constant (see also Table 21). On a different day the recovery rate of infectious virus after treatment with disinfectant was determined. The ratio of recovery rate: disinfectant over control was used as a measure of efficacy of disinfection.

For disinfection of poliovirus 1-contaminated hands 80% ethanol was little effective (Table 21), despite substantial activity in the suspension test (Table 3). This discrepancy has been described and analyzed before (22). Besides possible "sheltering"

effects of the skin evaporation of the alcoholic disinfectant may play a significant role. It has, however, likewise been observed with an aqueous iodine solution against rhinovirus type 29 on skin (fingers) (3).

Quite satisfactory results were obtained with Desderman against rotavirus on hands (Table 22). It should be pointed out that the recovery rate of rotavirus in the control experiments was fairly constant 20%. Differences in efficacy of disinfection within the same group, e.g. 5 min of disinfection, were due to variation in the amount of virus recovered after application of Desderman. The reasons for the different efficacies with the various subjects are not yet known. Studies are in progress to determine whether it is influenced in a systematic fashion by the individual.

Effective disinfection was achieved in practical periods of time. Even a 1 min treatment could be effective, but apparently it is too short to reach a certain margin of safety.

It is of interest that a 3x1 min treatment was not significantly more effective than a 3 min treatment, although in the first case a 3-fold amount of disinfectant had been applied. This finding appears to weaken the argument that evaporation might be a significant factor in this situation, as discussed above. It is, nevertheless, obvious that the suspension test also in this case yields more optimistic results than those achieved with disinfection of hands. However, a striking reduction of rotavirus on skin appears feasable and may contribute to the control of nosocomial rotavirus infections.

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Spectrum of Clinical Illness in Hospitalized Patients with "Common Cold" Virus Infections

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The viruses associated most frequently with the "common cold" are rhinoviruses and coronaviruses. The first prospective cohort study to determine the prevalence of rhinovirus and coronavirus infections in patients of all ages hospitalized for acute respiratory illnesses is described. Hospital admissions for acute respiratory illnesses were identified, and cell culture for rhinovirus and serologic assays on paired sera for coronaviruses 229E and OC43 were performed. A total of 61 infections with rhinoviruses and coronaviruses were identified from 1198 respiratory illnesses (5.1%); in addition, 9 additional infections associated with ≥1 other respiratory viruses were identified. Of those infected with only rhinovirus or coronavirus, underlying cardiopulmonary diseases were present in 35% of the patients aged <5 years, in 93% aged between 5 and 35 years, and in 73% aged >35 years. The predominant clinical syndromes varied by age: pneumonia and bronchiolitis in children aged <5 years; exacerbations of asthma in older children and young adults; and pneumonia and exacerbations of chronic obstructive pulmonary disease and congestive heart failure in older adults. Therefore, rhinovirus and coronavirus infections in hospitalized patients were associated with lower respiratory tract illnesses in all age groups.

Rhinoviruses and coronaviruses are the most frequently identified causes of the "common cold" syndrome [1–3]. Rhinoviruses are members of the *Picornaviridae* family and were first identified in 1956 [4]. Since then, >100 different serotypes have been identified [5]. Human coronaviruses, members of the Coronaviridae family, were first identified in 1962 and have been particularly difficult to isolate by use of standard cell culture techniques [6, 7]. A self-limited upper respiratory tract illness, or "common cold" syndrome, is the usual clinical manifestation of infection with these viruses. However, over the past 3 decades, several studies have found these viruses to be associated with clinical syndromes that require hospital care [8–19]. We recently performed a prospective, cohort study to evaluate the association of respiratory virus infection with respiratory conditions identified in hospitalized patients [20]. In this article, we describe the demographics and clinical characteristics of hospitalized patients identified with recent rhinovirus or coronavirus infection.

Methods

Study design. This prospective study was conducted in an urban public teaching hospital (Ben Taub General Hospital), a children's hospital (Texas Children's Hospital), and a community hospital (St. Luke's Episcopal Hospital) in Houston, Texas, from 1991 through 1995 [20]. Patients with an admitting diagnosis suggestive of an acute respiratory condition or congestive heart failure (CHF) were visited in the hospital to confirm eligibility and to seek consent for participation. Acute respiratory disease diagnoses included the following: pneumonia, tracheobronchitis, bronchitis, croup, and exacerbations of asthma or chronic obstructive pulmonary disease (COPD). Patients with active tuberculosis or known HIV disease were excluded. On admission to the hospital, a nasal and/or throat swab and an acute serum specimen were obtained. A convalescent serum was collected ≥10 days after obtaining the acute serum. Patients' inpatient medical records were reviewed. Information collected included sociodemographic data, symptoms, signs, acute respiratory diagnosis and other diagnoses, the location of admission (i.e., general ward or intensive care unit), the duration of hospitalization, the need for ventilatory support, and the chest radiograph interpretation.

Laboratory methods. Respiratory secretion specimens were inoculated on 4 cell culture lines, including human embryonic lung fibroblast cells (WI-38), continuous human epithelioid carcinoma cells (Hep-2), rhesus monkey kidney cells (LLC-MK2), and primary rhesus monkey kidney or Madin-Darby canine kidney (MDCK) cells. Rhinoviruses were identified initially by the characteristic cytopathic changes induced in cell culture. Rhinoviruses were differentiated from enteroviruses by use of acid lability or reverse transcriptase (RT)–PCR assays [21]. Coronavirus infections were identified serologically by use of paired serum samples, with the convalescent serum being collected at least 10 days after the acute serum collection. Antibody tests for coronavirus 229E were performed by use of a microneutralization assay, and infection was defined by the presence of a ≥4-fold increase in antibody levels [22]. An ELISA was used to measure antibodies to coronavirus

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OC43, and infection was defined as a ≥4-fold increase in ELISA IgG antibodies or by a ≥2.5 increase that was confirmed on a repeated test [23]. Coinfections with other respiratory viruses were identified by cell culture and serology, as described elsewhere [24].

Results

A total of 1068 patients were enrolled in the study, and these patients had 1198 illnesses evaluated. Of these illnesses, 408 (34.1%) had paired sera available for testing for coronavirus infection. Sixty-one illnesses were identified that were associated with a rhinovirus or coronavirus infection, but infection with no other respiratory viruses (table 1); 9 additional illnesses were associated with both a "common cold" virus (6 rhinoviruses and 3 coronavirus OC43) and an additional respiratory virus (influenza virus, respiratory syncytial virus [RSV], or parainfluenza virus). These patients with dual virus infections tended to be younger (5 of 9, aged <5 years) and more likely to have had paired sera collected (6 of 9 versus 18 of 61, respectively) than the group identified to have only "common cold" virus infections. These illnesses were not considered further because of the potential confounding effect of the additional respiratory virus infection.

Table 1. Sociodemographics and laboratory abnormalities in hospitalized patients with infections caused by rhinoviruses and coronaviruses.

Data	Age group, years		
	<5	5–35	>35
Total no. of illnesses evaluated	419	233	546
Total no of illnesses with paired sera available	83	99	226
No. of patients with "common cold" virus infection[a]	20	15	26
Rhinovirus	18	14	13
Coronavirus	2	1	13[b]
Male:Female	8:12	6:9	8:18
Race			
Black	4	7	14
Hispanic	15	4	4
White	1	3	8
Asian	0	1	0
History of smoking	0	2	18
History of asthma	5	13	18
Prior influenza vaccine	0	4	12
Prior pneumococcal vaccine	0	1	4
Mean no. in household	5.6	3.8	3.0
Mean WBC count, cells/μL	16,000	12,500	10,900
Positive blood cultures/total	2/9[c]	0/3	2/7[d]
Chest radiographic abnormalities	9/16	5/11	18/25

NOTE. RSV, respiratory syncytial virus.

[a] Does not include 9 dual infections: 5 in age group <5 years (2 rhinovirus/RSV, 1 rhinovirus/influenza A virus, 1 rhinovirus/RSV/influenza A virus, and 1 coronavirus OC43/influenza A virus coinfections), 3 in age group 5–35 years (2 rhinovirus/influenza A virus and 1 coronavirus OC43/influenza A virus coinfections), and 1 in age group >35 years (coronavirus/influenza B virus/parainfluenza type 3 virus coinfection).

[b] One dual infection (coronavirus OC43 and coronavirus 229E).

[c] Two positive for *Staphylococcus* species.

[d] Two positive for *Streptococcus pneumoniae*.

The racial/ethnic distribution of the patients with a "common cold" virus infection was similar to that of the overall study population: 41% (n = 25) versus 51% black; 38% (n = 23) versus 31% Hispanic; 17% (n = 12) versus 16% white; and 1% (n = 1) versus 1% Asian. In addition, the age distribution of patients with a rhinovirus or coronavirus infection was similar to that of the overall study population: 33% versus 37% aged <5 years, 25% versus 19% aged 5–35 years, and 43% versus 44% aged >35 years. Eighteen (90%) of the 20 patients aged <5 years, 14 (93%) of 15 patients between the ages of 5 and 35 years, and 13 (50%) of 26 patients aged >35 years had rhinovirus infections. The remaining 16 patients had coronavirus infections.

Illnesses associated with "common cold" viruses occurred throughout the year but were less frequent during summer months. The distribution of illnesses was as follows: 28% of cases occurred between January and March, 25% between April and June, 13% between July and September, and 34% between October and December. Fifty-eight percent of rhinovirus infections and 81% of coronavirus infections occurred between October and March; 8 of the 9 infections associated with other respiratory viruses also occurred during this period.

The patients' admitting diagnoses were tabulated (table 2). In the group aged <5 years, the most common clinical diagnoses on admission were asthma (n = 7), bronchiolitis (5), possible sepsis (5), and pneumonia (4). For patients between the ages of 5 and 35 years, the primary reason for admission was for the treatment of asthma (14); 1 patient had pneumonia. For patients aged >35 years, most clinical diagnoses on admission were for the treatment of pneumonia (n = 8) CHF (7), COPD (7), and asthma (5). Overall, a chronic cardiopulmonary disease diagnosis was present in 39 (64%) of 61 patients, with asthma being the most common illness, followed by CHF and COPD.

Hospital care took place on the wards in 66% of the cases; however, 34% of the cases were treated in the intensive care unit or the intermediate care unit. One patient aged <35 years required mechanical ventilation compared with 2 patients aged >35 years (table 3). Fever and an elevated WBC count were more common in patients aged <5 years, and wheezing was seen in all age groups. Two of 9 cultures of blood obtained from patients aged <5 years were positive for *Staphylococcus* species (probable contaminants). Two cultures of blood obtained from patients who were aged >35 years and had pneumonia were positive for *Streptococcus pneumoniae*. Antibiotics were prescribed for the majority of patients. All patients survived, and the mean (median) length of stay was 3.4 (3) days for patients aged 0–5 years; 3.5 (2) days for patients aged 5–35 years; and 6.0 (6) days for patients aged >35 years.

Changes seen on chest radiographs largely reflected the clinical diagnoses. For patients aged <5 years, abnormalities were reported in 9 (56%) of 16 patients (infiltrates or pneumonia, 6; hyperinflation, 2; and atelectasis, 1). Abnormalities were seen in 6 (55%) of 11 patients aged between 5 and 35 years (hyper-

Table 2. Spectrum of clinical illness in hospitalized patients with "common cold" virus infections.

Clinical diagnoses[a]	Age group, years <5 (*n* = 20)	5–35 (*n* = 15)	>35 (*n* = 26)
Asthma	7	14	5
Bronchiolitis	5	—	—
Congestive heart failure	—	—	7
Chronic obstructive pulmonary disease	—	—	7
Croup	1	—	—
Fever/conjunctivitis	1	—	—
Otitis media	2	—	—
Pneumonia	4	1	8
Possible sepsis	5	—	—
Upper respiratory infection	1	—	—

[a] Can have >1 diagnosis.

inflation, 4; and infiltrates, 2), and abnormalities were detected in 18 (72%) of 25 patients aged >35 years (pneumonia, 7; CHF/cardiomegaly, 5; emphysematous changes, 4; and pulmonary nodule, 2).

Discussion

Rhinoviruses and coronaviruses are usually recognized as causes of upper respiratory illnesses that are mostly benign and self-limited. However, these "common cold" viruses have also been associated with illnesses that require hospitalization. In previous reports, the role of one or both of these viruses has been evaluated in a select population, on the basis of age or underlying disease [8–19]. Our study is the first to analyze the clinical characteristics of patients from all age groups who were admitted with acute respiratory disease and were infected with a rhinovirus or coronavirus. We found that the distribution of presentations varied with the age and underlying disease of the patient. Only 35% of patients aged <5 years had underlying chronic pulmonary disease (asthma). In contrast, 93% of patients aged between 5 and 35 years had asthma, and 73% of patients aged >35 years had asthma, COPD, or CHF.

Bronchiolitis and pneumonia are the most common clinical syndromes associated with rhinovirus infection in hospitalized children aged <5 years [8–16], a finding confirmed by our study. RSV is the most frequently identified respiratory virus for which young children are hospitalized for an acute respiratory condition, and in some studies rhinovirus has been the second most common [10–13]. The clinical presentation of rhinovirus infection may be similar to that of RSV [15]. In addition, coronavirus infections have been associated with lower respiratory tract illness, including pneumonia and bronchiolitis, in children aged <5 years [19]. There is less information available regarding the relative importance of coronavirus infections in this age group. We identified only 2 coronavirus infections in patients aged <5 years, although paired sera were available for only ~20% of the patients in this age group.

The association of respiratory virus infections with exacerbations of asthma has been recognized for several decades [25–27]. In the last several years, respiratory virus infections have been identified in up to 80% of wheezing episodes in school-aged children and in >50% of wheezing episodes in adults [17, 28, 29]. Virus-associated exacerbations can be severe enough to require hospitalization [17, 18], and a correlation between hospitalizations for asthma and the seasonal prevalence of upper respiratory virus infections has been noted, especially in children. In several studies, rhinovirus is the most common respiratory virus associated with asthma exacerbations, and coronavirus is the second most frequent [17, 28, 29]. In the current study, the principal reason for hospitalization of older children and young adults with a rhinovirus or coronavirus infection was an asthma exacerbation.

Respiratory virus infections have also been associated with worsening of other chronic diseases, including cardiopulmonary disorders such as COPD and CHF [31–34]. Although the proportion of exacerbations of these diseases associated with respiratory virus exacerbations has been lower than that seen for asthma, the burden of these infections is substantially greater in chronically ill patients than in healthy subjects of a similar age [33, 34]. In the current study, many of the patients aged >35 years had underlying lung disease or CHF. In addition, several patients were admitted with pneumonia. It is unclear whether rhinovirus can cause pneumonia itself [35, 36]. It has been recovered from the lower respiratory tract of infected individuals [37, 38], and an increase in the number of inflammatory cells in the bronchial mucosa has been noted after experimental infection of the upper respiratory tract [39]. The possibility that these viruses might be associated with an increased risk of bacterial pneumonia, similar to that described for influenza [40, 41], is suggested by the identification of at least 2 pneumonia illnesses in the oldest age group associated with a concomitant *S. pneumoniae* infection. However, this study was not designed to delineate such a virus-bacteria interaction.

There are several potential shortcomings of the current study. Paired sera were available for only a subset of the population; thus, there is substantial potential for the introduction of a bias in identifying the relative importance of coronavirus infections in this population. On the other hand, infections with additional

Table 3. Clinical features and medical care requirements for patients with infections caused by "common cold" viruses.

Data	Age group, years <5	5–35	>35
Mean (median) duration of hospitalization, days	3.8 (3)	3.5 (2)	6.0 (6)
Admission temperature ≥38°C	55	27	15
Wheezing on admission	45	87	50
ICU/IMC care	30	60	23
Supplemental oxygen	25	73	77
Mechanical ventilation	0	7	8
Antibiotics administered	70	40	85

NOTE. Data are percents unless indicated otherwise. ICU, intensive care unit; IMC, intermediate care unit.

respiratory viruses, such as influenza virus or RSV, may have been missed in those persons who did not have serologic studies performed. Another potential shortcoming is that control populations were not included in the study; therefore, the frequency of rhinovirus or coronavirus infections in ambulatory populations, in populations hospitalized with nonrespiratory conditions, or in asymptomatic populations cannot be determined. However, in a study for which virologic studies were performed in the same laboratory during the same period, <2% of cultures were positive for any respiratory virus from an asymptomatic adult and <1% of serologic studies identified a respiratory virus infection during an asymptomatic period [33]. The use of RT-PCR assays for rhinovirus and coronavirus has increased the frequency with which these virus infections were identified, compared with cell culture or serologic methods in previous studies of asthmatic patients [17, 28, 29]. Such assays were not performed in the current study; therefore, the burden of these infections detected in this study is likely to be a minimal estimate of their infection frequency.

The morbidity associated with rhinovirus and coronavirus infections, especially in high-risk populations such as patients with chronic lung disease, suggests that these infections should be a target for prevention or treatment strategies. The development of a conventional vaccine for rhinovirus has been hampered by the existence of >100 different serotypes and by the lack of protection associated with experimental parenteral vaccines [42]. Less is known about the number of coronavirus serotypes, and no experimental human vaccines have been evaluated. IFN-α is an antiviral agent that has been effective in both experimental rhinovirus and coronavirus infection [43–45], and also has been used effectively as postexposure prophylaxis for natural colds [46, 47]. The utility of IFN-α in normal healthy populations has been limited by its local (nasal) toxicity. Only a single study has evaluated its efficacy for the prevention of respiratory virus infections and the resulting complications in patients with chronic lung disease [48]. No beneficial effects of IFN-α were seen in this population of patients with asthma and COPD; however, the study had insufficient power to demonstrate an effect caused by the prevention of rhinovirus and coronavirus infections. Newer antirhinovirus drugs, such as tremacamra and pleconaril, have recently been shown to have beneficial effects in the treatment of experimental rhinovirus infection [49, 50], which suggests that there may be a role for these drugs in high-risk populations. Because of the morbidity associated with rhinovirus and coronavirus infections in high-risk patients, these groups should be targeted in future evaluations of antiviral chemotherapy strategies.

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Micro-organisms in gastroenteritis

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SUMMARY We present bacteriological and virological findings together with salient clinical features from a prospective study of 447 children aged under 2 years admitted to hospital with infectious gastroenteritis. Putative pathogenic micro-organisms were identified in the stools of 75% of these children. Eight identifiably distinct groups of viruses, found on electron microscopy and tissue culture were present in 67% of patients—rotavirus was detected most frequently. Pathogenic bacteria (salmonellas, shigellas, *Escherichia coli,* and *Campylobacter jejuni*—but excluding *Clostridium difficile*) were found in 16% only. Altogether 4·9% of 390 patients had gastroenteritis associated with *Cl difficile* toxin.

The mean duration of diarrhoea was shortest in patients with identifiable virus, with rotavirus having a mean of 5·01 days, and was longest in patients with pathogenic bacteria in the stools (11·14 days). The finding of more than one type of virus did not seem to be associated with a significantly increased duration of diarrhoea. There are few clinical features which can be associated specifically with any particular micro-organism or groups of these. Multiple organism isolation was common, but the severity of the illness in those patients with at least two types of organism was not greater. Certain viruses, including the norwalk-like virus, known to be associated with outbreaks of gastroenteritis were found as frequently in a group of patients who did not have diarrhoea studied for comparison. Virus was still detectable in the stools of up to 40% of asymptomatic children on the day of discharge.

In 1967 a major study by Ironside *et al*[1] from this unit showed that pathogenic organisms could be identified in only 16% of children aged under 2 years admitted with infectious gastroenteritis. Since that time there have been major advances in both virological (mainly electron microscopy and tissue culture techniques) and bacteriological laboratory techniques, with the identification of several new pathogens. It was appropriate, therefore, to carry out a modern study in the same unit.

Patients and methods

The Regional Infectious Diseases Unit at Monsall Hospital serves a population of 1¼ million people drawn from a mixed racial, industrial and business population mainly from the north of Greater Manchester.[2]

Children aged less than 2 years who were admitted with acute diarrhoea (frequent watery or unformed offensive stools) with or without vomiting, judged to be caused by primary infectious gastroenteritis were entered into this prospective study over the 12 month period December 1981 to November 1982. The referring general practitioner was briefly questioned about the child's illness, drugs prescribed, and reason for hospital admission. Mother was interviewed within 24 hours of admission and a detailed questionnaire relating to the management and course of the illness before hospital admission was completed. Clinical examination of the child was followed by appropriate treatment and the child's status was monitored until discharge, when he or she was asymptomatic. Assessment of dehydration was based on the Medical Research Council criteria previously described.[1]

A sample of faeces was obtained on admission to hospital and again on the day of discharge (when symptom free). Routine phlebotomy was performed for full blood count, serum electrolytes, and urea. Blood cultures, throat swab, and midstream urine sample were taken for bacteriology.

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Results

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There were 447
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Stools were routinely cultured for enteropathogenic *Escherichia coli*, salmonellas, campylobacter, shigellas, and yersinia; they were examined microscopically for ova, cysts, and parasites. The technique used for isolation of *Clostridium difficile* and detection of toxin has been described elsewhere.[3]

Preparation of faecal specimens for electron microscopy. All faecal specimens were stored at 4°C. Twenty per cent faecal suspensions in faecal transport medium were centrifuged at 1500 *g* for 15 minutes at 8°C (clarification spin). Supernatant (2 ml) was transferred to a polycarbonate ultracentrifuge tube and spun at 65 000 *g* for one hour at 8°C. The supernatant from the ultracentrifuge spin was tipped off and the pellet resuspended in the small amount of fluid remaining. Viruses were adsorbed onto Formvar-carbon coated electron microscope specimen grids, stained with 3% PTA (pH 6 to 6·5), and examined at 63 000 magnifications in an AEI (Kratos) EM801 electron microscope.

Tissue culture technique. A thawed 20% faecal emulsion (0·1 ml) was inoculated into three cell lines: primary baboon kidney (BK), diploid fibroblasts (MRC 5), and continuous human epithelial cells (HEp 2). These were examined twice a week for evidence of cytopathic effect and were discarded after two weeks.

For comparison, all other children aged under 2 years and admitted over the same period with non-gastrointestinal illness had their faeces examined as above. The most common diagnosis in the comparison group was that of a respiratory illness—whooping cough being most frequent.

Statistical analysis. Fisher's exact test and Student's *t* test were used as appropriate.

Results

The reasons for admission to hospital given by the referring doctor included: (1) dehydration (11%); (2) failure of symptoms to settle on home management (52%); (3) adverse social factors (20%); (4) for isolation (12%); and (5) poor general condition (7%). No specific reason was given in 11% of the children.

There were 447 hospital admissions (including 21 children with two admissions); the boy:girl ratio was 1·36; and most children were aged under 1 year (Table 1; Fig. 1). There were 162 children in the comparison group, most of whom had a respiratory infection including whooping cough (117). The most common diagnoses in the remainder were measles, chickenpox, meningitis, or miscellaneous dermatological disorders.

Table 1 *Prevalence of viruses and bacteria*

	Gastroenteritis group	*Comparison group*
No	447	162
Boy:girl	1·36	1·08
One or more agents* (no (%))	335 (75)	70 (43)
Two or more agents (no (%))	127 (28)	14 (9)
Viruses *only* (no (%))	257 (57·5)	70 (43)
Pathogenic bacteria *only* (no (%)	29 (6·5)	0 (0)
Viruses *and* bacteria (no (%))	43 (10)	0 (0)
Clostridium difficile toxin *only* (no (%))	6 (1)	0 (0)
No agent* (no (%))	112 (25)	92 (57)
Clostridium difficile isolated (no (%))	219/447 (49)	78/118 (66)
Clostridium difficile toxin (no (%))	19/390 (4·9)	3/118 (1·8)

*Agent=potential viral or bacterial pathogen.

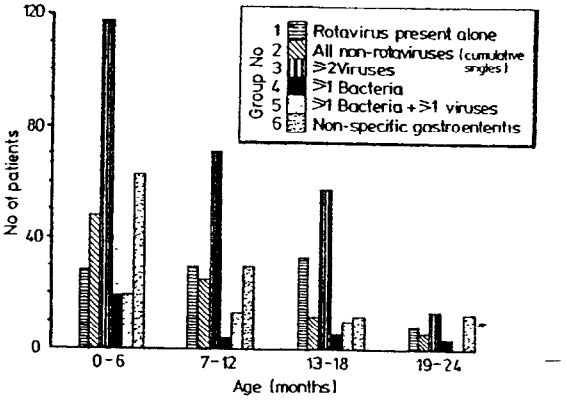

Fig. 1 *Age distribution of micro-organisms found in gastroenteritis patients.*

See text for definition of groups.

There was no significant difference between patients with gastroenteritis and those in the comparison group in the delay between stool collection and laboratory examination—77% of all stool specimens collected were examined at 24 hours after collection. Seventy five per cent of the children with gastroenteritis had at least one micro-organism present in the stool—57·5% had viruses only, 6·5% had bacteria only, and 10% had both bacteria and viruses. No patient had parasites. Twenty five per cent of children had no identifiable pathogenic bacteria or viruses. *Cl difficile* was present in 49%. *Cl difficile* toxin was found in 19 of 390 patients (4·9%), of whom six had no other bacteria or viruses. Details of these findings relating to *Cl difficile* and its toxin are published separately.[2] Thirty eight per cent of those children who had

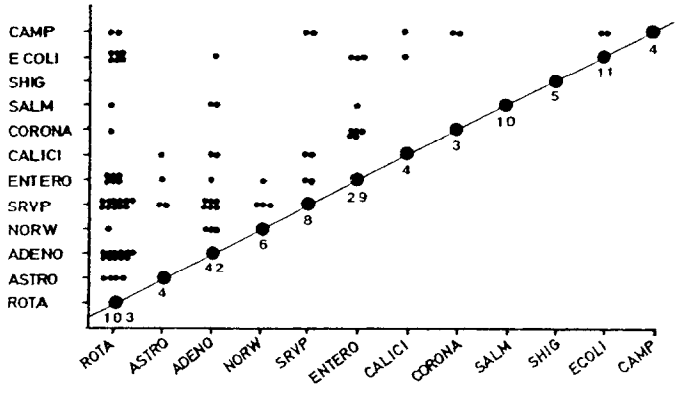

Fig. 2 *Association of micro-organism isolates. 'Pure' single isolates shown along the diagonal.*

Camp=*Campylobacter jejuni*
E coli=enteropathogenic *Escherichia coli*
Shig=shigellas
Salm=salmonellas
Corona=coronavirus
Calici=calicivirus
Entero=enterovirus
SRVP=small round structureless virus particles
Norw=norwalk-like virus
Astro=astrovirus
Rota=rotavirus
Adeno=adenovirus

pathogenic micro-organisms had at least two concurrently, and in seven per cent there were at least three (Table 1, Figs. 2 and 3).

Several morphologically distinctive viruses were identified, namely: rotavirus, adenovirus, norwalk-like virus, calicivirus, coronavirus, astrovirus, culturable enterovirus (including echovirus, coxsackievirus, and untypable (through unavailability of antisera)), and a heterogeneous group including parvovirus, picornovirus, and some non-culturable enteroviruses—the 'small round structureless virus particles'.[3–5]

Rotavirus was the most commonly identified organism (153 of 447 patients: 34%); adenovirus and enterovirus were found in 17·2% and 12·5% respectively. Astroviruses, norwalk-like viruses, caliciviruses, coronaviruses, and small round structureless virus particles collectively were found in 25%. *E coli* and *Campylobacter jejuni* were isolated from 6·9% and 5·1% of patients respectively: apart from *Cl difficile* these were the two most common pathogenic bacteria (Table 2).

Rotavirus, adenovirus, enterovirus, salmonellas, and shigellas were the five organisms which were usually found alone. The remainder were found more often in combination with others, in particular the small round structureless virus particles, astrovirus, and *C jejuni* (Fig. 2).

The adenoviruses found in association with gastroenteritis were usually identified by electron microscopy but either failed to grow or proved untypable on tissue culture (55 of the 85 patients). Table 3

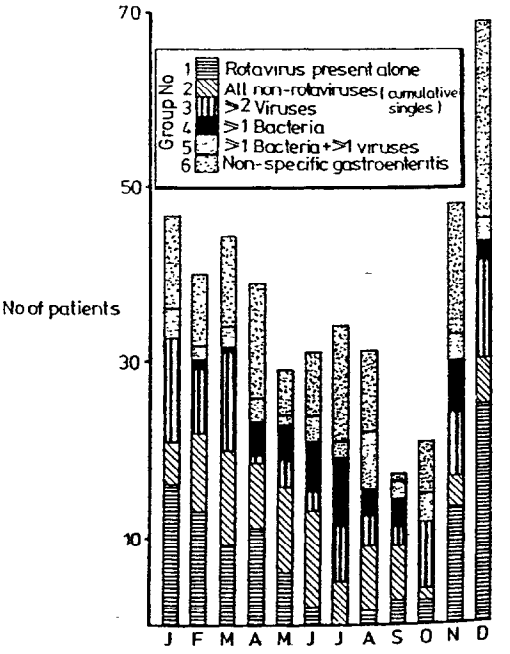

Fig. 3 *Distribution of groups of gastroenteritis patients by month of admission to hospital.*

See text for definition of groups.

Table 2 *Micro-organisms found in patients*

Organism	*Gastroenteritis patients with organism (no (%))*	*Comparison group patients with organism (no (%))*	*P value*
Rotavirus*	153 (34)	7 (4·3)	<0·0001
Adenovirus*†‡	77 (17·2)	15 (9·3)	<0·01
Enterovirus†	56 (12·5)	15 (9·3)	NS
Norwalk-like virus*	16 (3·6)	6 (3·7)	NS
Calicivirus*	16 (3·6)	0 (0)	<0·01
Coronavirus*	11 (2·5)	2 (1·2)	NS
Astrovirus*	16 (3·6)	2 (1·2)	NS
Small round structureless virus particles*§	44 (9·8)	19 (11·7)	NS
Salmonellas	19 (4·3)	0 (0)	
Shigellas	9 (2)	0 (0)	
Escherichia coli	31 (6·9)	0 (0)	
Campylobacter jejuni	23 (5·1)	0 (0)	
None	112 (25)	92 (57)	

*Identified by electron microscopy, †identified by tissue culture. ‡See Table 3 for breakdown, §see text for definition.

Table 3 *Details of adenoviruses found and associated respiratory infection*

	Gastroenteritis patients No (%)	*Comparison group patients No (%)*
Identified by tissue culture and typable (group A)	30 (35)*	14 (78)
Identified by tissue culture and untypable (group B)	38 (45)†	2 (11)
Identified by electron microscopy alone (group C)	17 (20)	2 (11)
Total	85 (100)	18 (100)
Associated respiratory infection	14 (16)‡	13 (72)‡
Serotypes group A type 1	5	3
2	16	0
3	2	0
4	1	0
5	0	2
6	2	0
7	0	7
Others	4	2

*20 had adenovirus also seen by electron microscopy, which may not have been the same virus as that identified by tissue culture
†35 had adenovirus also seen by electron microscopy, which may not have been the same virus as that identified by tissue culture
‡Significant difference P<0·001

gives details of the adenoviruses found. Thirty were typable (mainly type 2). This contrasts with findings in the comparison group where 14 of the 18 were typable, and these patients had a significantly higher prevalence of respiratory symptoms (72%) compared with the gastroenteritis patients (16%).

In the comparison group, all organisms were found significantly less often apart from the norwalk-like virus, small round structureless virus particles, coronavirus, astrovirus, and enteroviruses (Table 2). In none of these patients were pathogenic bacteria, apart from *Cl difficile*, isolated. *Cl difficile* toxin was present in three comparison group patients.

Patients with gastroenteritis were divided into six groups, according to the micro-organisms isolated, thus permitting a comparison of the main clinical and biochemical features. Group 1 comprised patients in whom rotavirus was present alone; group 2, those in whom only any one virus—not rotavirus—was isolated, expressed as cumulative singles; group 3, patients in whom two or more viruses were isolated; group 4, those in whom one or more bacteria were present, alone or in combination; group 5, patients with one or more bacteria plus any one or more viruses in combination; and group 6 comprised patients in whom no pathogenic bacteria or viruses were present. *Cl difficile* was isolated from all six groups. These findings are summarised in Table 4. Patients excreting any virus tended to present during the winter months; those excreting bacteria presented during the warmer season (Fig. 3).

Diarrhoea persisted longer than vomiting in all groups. The mean duration was shortest in those with rotavirus alone (5·01 days) and longest with bacterial isolates (11·14 days). Viruses other than rotavirus tended to produce a significantly longer duration of diarrhoea (7·05 days) but the simultaneous finding of at least two different types of viruses was not associated with longer duration of diarrhoea (7·23 days). Furthermore, the presence of virus and bacteria did not seem to alter the duration of diarrhoeal symptoms (10·81 days) when compared with patients in whom bacteria alone were found (11·14 days). The non-specific gastroenteritis group seemed to have less diarrhoea than the bacteria groups and approximated to that for viruses, with a mean duration of 6·60 days.

Duration of vomiting in all groups ranged from 2·21 to 3·65 days, viruses tending to have a longer duration of symptoms than bacteria—these differences, however, were not significant, apart from the group with bacteria and viruses in whom the duration (2·21 days) was significantly less than with rotavirus alone.

Moderate to severe dehydration occurred in 14% of patients but acidosis was not a frequent finding. Hypernatraemia was found in less than one per cent and in patients excreting virus alone.

Of other associated clinical findings, the prevalence of stool mucus was significantly increased in viruses other than rotaviruses and in the group with bacteria and virus found together. Macroscopic blood in the stool was highly significantly increased in patients with bacteria compared with rotavirus. Lower respiratory infection occurred in up to 21% of patients and its prevalence was significantly

Table 4 *Clinical and other features in 447 gastroenteritis patients classified into six major groups by micro-organisms, showing significant differences from group 1 in which rotavirus was found alone*

	Group					
	1 Rotavirus alone	*2 All viruses except rotavirus (cumulative singles)*	*3 Combination of viruses*	*4 All bacteria*	*5 Bacteria and viruses*	*6 Non-specific gastroenteritis*
No	100	87	70	29	43	118
Duration of diarrhoea (days) Mean (SEM)	5·01 (0·53)	7·05 (1·03)[c]	7·23 (0·66)[a]	11·14 (1·35)[d]	10·81 (1·74)[d]	6·6 (0·58)[b]
Duration of vomiting (days) Mean (SEM)	3·53 (0·37)	3·65 (0·8) ns	3·13 (0·43) ns	2·45 (0·79) ns	2·21 (0·37)[b]	2·91 (0·3) ns
Mucus present in stools No (%)	13 (13)	21 (25)[e]	17 (24) ns	11 (38) ns	13 (30)[e]	24 (20) ns
Blood present in stools. No (%)	3 (3)	8 (10) ns	1 (1) ns	12 (41)[g]	14 (33)[g]	5 (4) ns
Lower respiratory tract infection. No (%)	10 (10)	18 (21) ns (adeno[f])	10 (15) ns	4 (14) ns	5 (12) ns	18 (15) ns
Urea >6 mmol/l No (%)	17 (17)	10 (12) ns	6 (9) ns	0 (0)[f]	0 (0)[f]	9 (8) ns
White cell count >13×10⁹/l No (%)	18 (18)	37 (43)[g]	27 (39)[f]	15 (50)[f]	22 (50)[f]	55 (47)[g]
Hospital stay ≥ 7 days No (%)	24 (24)	26 (31) ns	27 (39)[e]	17 (59)[g]	22 (50)[f]	29 (25) ns

[a]P=0·005, [b]P=0·025; [c]P=0·05, [d]P=0·0005 (Student's *t* test).
[e]P<0·05; [f]P<0·025, [g]P<0·01, [h]P<0·001 (Fisher's exact test).
ns=no significant difference from group 1
See text for fuller definition of groups

increased in patients with adenovirus infections. A greater proportion of patients with viruses and with non-specific gastroenteritis had a higher blood urea concentration compared with those with bacteria. The proportion of patients with a peripheral white cell count over 13×10^6/l was significantly greater in all groups compared with the rotavirus group.

The duration of hospital stay was determined primarily by whether symptoms had settled and not by continuing faecal excretion of the organism. Seventy six per cent of patients with rota virus and 75% patients with non-specific gastroenteritis had a short hospital stay (less than seven days); in those with at least two viral agents the stay was longer, and in those with bacteria more than 50% remained in hospital for over seven days.

There were no other significant differences in the clinical and biochemical features between the six groups (Table 5). In particular, the patient's general condition was not more severe in those who had at least two micro-organisms present.

Ten children on admission and a further 20 within 48 hours of this (six per cent in total) required intravenous fluid replacement for one to two days in preference to oral rehydration treatment, to correct dehydration or to overcome persistent or severe symptoms. Most, including many with moderate to severe dehydration, were successfully rehydrated with a standard regimen of sodium chloride and glucose solution (Dioralyte, Armour Pharmaceutical) for 24 hours combined with temporary food and milk withdrawal, followed by a graduated

Table 5 *Incidence of clinical and other features not showing any significant differences between the rotavirus group (group 1) and the other groups of patients with gastroenteritis (groups 2–6)*

Feature	*Overall incidence (no (%))*
Upper respiratory infection	80 (18)
Otitis media	116 (26)
Appreciable lymphadenopathy	94 (21) (adenovirus 16%)
Maculopapular rash	58 (13) (adenovirus 11%)
Convulsions	5 (1)
General condition well	290 (65)
Projectile vomiting	170 (38)
Temperature ≥38·5°C	36 (8)
Moderate to severe dehydration	64 (14)
Bicarbonate ≤15 mmol/l	13 (3)
Sodium ≥150 mmol/l	5 (1)
Total complications	89 (20)
Secondary lactose intolerance	36 (8)

dilutional re-introduction of either normal milk feeds or solid diet as appropriate.

Antibiotics were not routinely prescribed for gastroenteritis in this unit; only 16 patients received antimicrobial treatment as dictated by their clinical condition (salmonellas (five); *C jejuni* (two); *shigellas* (four); enteropathogenic *E coli* (one); *Cl difficile* colitis (six)).

There were no deaths during the study period, but complications were recorded in 85 patients. Five children had a convulsion before hospital admission; four of these had a fever in excess of 39·5°C on admission and this may have been the cause. None

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of these five children was severely dehydrated or hypernatraemic. The associated micro-organisms were: rotavirus (three) and shigella (one). There were no previous disposing central nervous system factors in these patients. One other child sustained a brief convulsion after admission—he had gastroenteritis associated with rotavirus, was severely dehydrated on admission (sodium 164 mmol/l; urea 21 mmol/l), but made a full and complete recovery. Neither the type of intravenous fluid nor the rate of replacement was felt to have been contributory to this child's convulsion. The more common complications were temporary secondary lactose intolerance resulting in a recrudescence of diarrhoea and necessitating the withdrawal of lactose-containing milk feeds.

Those patients who did not prove to have any pathogenic micro-organisms in their faeces did not seem to show any significant difference in the time that solid feeding had first been introduced or in the incidence of breast feeding.

Discussion

A decade ago the most commonly isolated micro-organism in infantile gastroenteritis was the enteropathogenic *E coli*, accounting for 11 to 16% of cases.[1] In the vast majority of children, no pathogens were isolated (non-specific gastroenteritis). Our study indicates that the incidence of non-specific gastroenteritis is now much less common, but still accounts for a considerable core of patients. The reason for this changed pattern is the discovery of several new pathogens which cause human diarrhoeal disease.

Most of the micro-organisms identified were viruses, among which the rotavirus was the most common. Individual bacteria made a small overall contribution; *C jejuni* is now included among these.

The association of *Cl difficile* toxin with human diarrhoea has only recently been appreciated.[6] In this study it was felt to have been the major factor in 19 patients, a frequency comparable with other established bacterial pathogens. The role of *Cl difficile* is detailed in another paper.[2]

It is of some interest that in many instances the simultaneous presence of more than one agent occurred in the same patient; this was most notable in the patients excreting *C jejuni, E coli,* small round structureless virus particles, calicivirus, and coronavirus. This phenomenon of multiple organism isolation makes assessment of the contribution of each micro-organism to the illness difficult. There was, however, no difference in disease severity between those who excreted a single organism and those with at least two. The occasional reported finding, therefore, that those patients who have more than one micro-organism may have more severe disease[7 8] does not seem to be a general phenomenon.

Numerous studies have established the role of rotavirus as an important human pathogen but the role of the newer viruses is uncertain. Viruses resembling the norwalk agent (norwalk-like viruses)[9] have been previously identified with some outbreaks of gastroenteritis, usually in older children and adults. Their low and equal prevalence in patients and in the comparison group reinforces the view that it is not an important cause of sporadic diarrhoea among infants. Although coronaviruses[4] have also been incriminated previously, their role is very debatable and this is also supported by our findings. Nevertheless, they may constitute an important community reservoir from which outbreaks may arise, given favourable conditions. On the contrary, all the caliciviruses and astroviruses, though small in number, were found almost without exception in patients with gastroenteritis, strengthening the view that they are pathogenic. These latter two viruses are not usually associated with such a young age group, however, occurring more often in older children.[10 11]

Thirty patients had culturable and typable adenoviruses in their stools, mainly of serotype 2 (group A, Table 3). Thirty eight patients had non-typable or poorly growing adenovirus (group B)—they may not have grown because of their fastidious nature, insufficient faecal concentration, or unavailability of specific antisera. Fifty five of these 68 patients (groups A and B) had adenovirus identifiable by electron microscopy as well, but it is uncertain whether the adenovirus seen by electron microscopy was the same as the one cultured. In the remaining 17 patients (group C) adenovirus was not culturable but was detected by electron microscopy alone. In contrast, most adenoviruses found in comparison group patients grew and were typable—mainly serotype 7. The relevance of these findings is not entirely clear but it is likely that adenoviruses found in group C were responsible for gastroenteritis, those in group B are more dubious, and those in group A unlikely. Our findings support the work of others[12 13] who argue that the adenoviruses associated with primary gastroenteritis are distinct from those associated with primary extragastrointestinal illness. There may well be other adenoviruses responsible for gastroenteritis which, owing to their fastidious nature, fail to grow under our tissue culture conditions, and these remain unrecognised.

A large proportion of children were discharged asymptomatic as convalescent excretors. This may

be of public concern and is at variance with the findings of others, who report a much lower percentage of children still excreting virus at this stage of convalescence.[14]

Overall, the clinical features indicate that gastroenteritis in this age group is nowadays a relatively benign self-limiting illness associated with a short stay in hospital and few complications. Apart from one child, convulsions occurred before admission; in only one child did there seem to be the precipitating factor of hypernatraemia. This is in striking contrast to the situation described a decade ago from this unit, when hypernatraemic dehydration associated with cerebral disturbance and related to high solute milk feeds and concentrated glucose drinks was common and carried an appreciable mortality.[1 15] This important aspect of management is discussed in detail elsewhere.[16]

The presence of macroscopic blood in the stools, a normal plasma urea concentration, a peripheral white cell count greater than 13×10^6/l, longer duration of diarrhoeal symptoms, and a longer stay in hospital all tend to suggest a bacterial rather than a viral aetiology for the gastroenteritis. This is not absolute, however, and there were no specific or characteristic clinical, biochemical, or haematological features in any particular group to indicate unequivocally a particular agent. Thus, for example, the widely held view that adenovirus infections are suggested by the presence of lymphadenopathy and a maculopapular rash seems untenable from our findings (Table 5). Also, our results indicate that it is not possible to make an emphatic diagnosis of rotavirus diarrhoea or rotavirus syndrome on clinical findings alone,[17] since upper and lower respiratory infection and otitis media were not found more commonly in those subsequently shown to have faecal rotavirus.

The group with gastroenteritis in whom no organisms were identified merits particular comment. This group was not associated with a higher incidence of extragastrointestinal features (otitis media, urinary infections etc) so that a 'parenteral' aetiology is unlikely, there was no increased prevalence of antibiotic usage in this group (16%) compared with the other groups (9 to 24%), and there was no increased delay in stool analysis excluding virological 'fall off'. Recent change in bowel flora precipitated by recent alterations in feeding schedules ('weanling diarrhoea') or a change from breast to bottle feeding were no more common in this group—hence acute alterations in bowel flora were unlikely to be the cause. On the other hand, the seasonal and age distribution together with the broadly similar clinical features of these patients compared with those who had an identifiable viral agent suggests a viral aetiology. It may be that some of these patients were 'missed' cases of rotavirus, adenovirus, or other viral gastroenteritis since electron microscopy is relatively insensitive[18] and existing tissue culture techniques may not identify some viruses, notably the enteric adenoviruses. Electron microscopy, however, is the only 'catch all' method currently available for the identification of viral associated gastroenteritis. The possibility is that some other agent, as yet undiscovered, was responsible.

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PCR and Restriction Endonuclease Analysis for Rapid Identification of Human Adenovirus Subgenera

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Subgenus identification of adenoviruses is of clinical importance and is as informative as identification by serotype in most clinical situations. A PCR-based identification of adenovirus subgenera A, B, C, D, E, and F and sometimes serotypes is described. The PCR uses nonnested primer pair ADRJC1-ADRJC2, which targets a highly conserved region of the adenovirus hexon gene, has a sensitivity of 10 to 40 copies of adenovirus type 2 (Ad2) DNA, and generates 140-bp PCR products from adenovirus serotypes representative of all the subgroups. The PCR products of all subgroups can be differentiated on the basis of the restriction fragment patterns produced by a total of five restriction endonucleases. In addition, serotypes Ad40 and Ad41 (subgroup F) and important serotypes of subgroup D (Ad8, Ad10, Ad19, and Ad37) can easily be differentiated, but serotypes within subgroups B and C cannot. The method was assessed by blind subgenus identification of 56 miscellaneous clinical isolates of adenoviruses. The identities of these isolates at the subgenus level by the PCR correlated 91% (51 of 56) with the results of serotyping by the neutralization test, and 9% (5 of 56) of clinical isolates produced discordant results.

Adenoviruses are double-stranded DNA viruses that are conventionally classified according to serotype (1 to 49) and subgenus (A to F) based upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis of virion polypeptides and restriction endonuclease (RE) analysis of the whole genome (40). Identification of these subgroups or serotypes can be of both clinical and epidemiological importance (21). Serotypes of subgenus A are isolated almost exclusively from the gastrointestinal tract (34). Adenoviruses of subgroup B, such as adenovirus type 3 (Ad3) and Ad7, and subgroup C (Ad1, Ad2, and Ad5) are common causes of respiratory tract infections (34, 39). Infections with these serotypes may persist asymptomatically for years in children, with the virus being shed continuously in the feces for many months after initial infection and intermittently for years thereafter (15). Certain members of subgroup D (Ad8, Ad19, and Ad37) cause outbreaks of conjunctivitis, and rapid identification of these serotypes can help in prevention and control (14). Subgroup E has one member, Ad4, which can cause either respiratory or eye infection, but a genotype variant of Ad4 (Ad4a) has been associated with outbreaks of conjunctivitis (39). Infantile gastroenteritis is caused by Ad40 and Ad41 (subgenus F) (5). In addition, fatal infections due to certain serotypes, such as those of subgroup B, have been reported (26, 34, 43).

Identification of adenovirus subgroups or serotypes can be achieved, with different degrees of efficiency, by serotype-specific neutralization tests (NTs) (16), RE analysis of DNA extracted from infected cells (40), and, more rarely nowadays, the hemagglutination inhibition test. The results of these methods, although of epidemiological value, are often of limited clinical usefulness. Up to 30 days may be required for complete characterization following the initial isolation of adenovirus in cell culture, which may itself require 30 days or more. In addition, certain adenoviruses such as Ad8, Ad40, and Ad41 are fastidious, with slow and inefficient growth in cell culture (13, 41). Alternative identification methods have therefore been developed and include the use of serotype-specific monoclonal antibodies (1, 42), detection of subgenus-specific antibodies (4), and PCR-based identification protocols (2, 4, 5, 18, 21, 28, 29, 30, 33). In this paper, we describe the development of a simplified, rapid PCR-based method for the identification of human adenoviruses at the subgenus level and, in some cases, the serotype level.

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MATERIALS AND METHODS

Extraction of DNA from virus isolates. Clinical isolates of Ad types 1 to 12, 14, 16, 19, 21, 31, 37, 40, and 41 typed by NT assay, RE analysis, or type-specific PCR (6, 20) were obtained from the Clinical Virology Laboratory, Manchester Royal Infirmary, Manchester, United Kingdom. DNA was extracted by the guanidinium thiocyanate (GuSCN) procedure described previously (7). Briefly, 200 μl of lysis buffer (4 M GuSCN, 0.5% *N*-lauroyl sarcosine, 1 mM dithiothreitol, 25 mM sodium citrate, 20 μg of glycogen) was mixed with 50 μl of infected-cell culture fluid (or sterile distilled water for an extraction-negative control), and the mixture was incubated at room temperature for 10 min, followed by addition of 25 μl of 3 M sodium acetate. The DNA was precipitated with 250 μl of ice-cold isopropanol, and the mixture was centrifuged at 12,000 × *g* for 10 min. The supernatant was discarded, and 500 μl of cold 70% ethanol was added, followed by centrifugation at 12,000 × *g* for 10 min. Ethanol was gently aspirated, and the pellet was dried in air before it was dissolved in 50 μl of Tris-EDTA buffer.

PCR. Under strict laboratory practice to avoid cross-contamination and carryover (23), the primer pair ADRJC1-ADRJC2 was used to amplify a 140-bp PCR product as described previously (11). The reaction mixture contained 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% (wt/vol) gelatin, 1.25 U of Amplitaq DNA polymerase (Perkin-Elmer Ltd., Warrington, United Kingdom), each deoxynucleoside triphosphate at a concentration of 200 μM, 0.2 μM each primer, and 5 μl of appropriate DNA sample or sterile distilled water as a contamination control in a total volume of 50 μl. The reaction was overlaid with 2 drops of mineral oil to prevent evaporation. Amplification was performed on a PHC-1 thermal cycler (Techne Ltd., Cambridge, England) by using one cycle of 94°C for 7 min, 55°C for 1 min, and 72°C for 1.5 min, followed by 40 cycles each of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min. The PCR products were analyzed with 8% polyacrylamide gels.

RE analysis of PCR products. The 140-bp PCR products generated from clinical isolates were digested with the REs *Taq*I, *Avi*II, and *Aat*II (all from Roche Diagnostics Ltd., Lewes, United Kingdom) and *Bse*RI and *Mnl*I (both from New England BioLabs Incorporated, Hitchin, United Kingdom). In a total volume of 20 μl, all reaction mixtures were prepared as recommended by the manufacturers, and those with REs *Taq*I, *Avi*II, and *Aat*II were incubated for 3 h

at the appropriate temperature and those with REs *Mnl*I and *Bse*RI were incubated overnight at the appropriate temperature.

Construction of plasmids and DNA sequencing. PCR products from Ad2, Ad8, Ad19, and Ad37 were cloned into the PCR-TOPO vector with the TOPO TA cloning kit (Invitrogen BV, Leek, The Netherlands) as described by the manufacturer. Recombinant plasmids were purified by the QIAGEN Plasmid Purification Maxi kit (QIAGEN Ltd., West Sussex, United Kingdom) and were sequenced by using the ABI Prism BigDye terminator cycle sequencing ready reaction kit (Perkin-Elmer Ltd.) and an automated sequencer (ABI 377; Perkin-Elmer Ltd). The nucleotide sequences obtained were aligned with sequences in the databases of the National Center for Biotechnology Information by using the Basic Local Alignment Search Tool family of programs, and RE analysis was performed with the software WebCutter, version 2 0.

Nucleotide sequence accession numbers. The nucleotide sequence accession numbers for all the sequences referred to in this paper are given in Fig. 1.

RESULTS

Sensitivities and specificities of primers. The primer pair ADRJC1-ADRJC2 has a detection limit of 10 to 40 copies of Ad2 DNA. The sequences of the upstream primer (ADRJC1) and the downstream primer (ADRJC2) were derived from the highly conserved DNA region which codes for the carboxy end of the monomeric protein II that forms the trimeric pseudohexagonal base of the adenovirus hexon. Both primers contain a maximum of two deliberately introduced mismatches compared with the sequences of the hexon genes of Ad2, Ad3, Ad4, Ad5, Ad7, and Ad16 and a maximum of four mismatches compared with the sequences of the hexon genes of the other serotypes. These mismatches did not involve the 3′ termini of the primers, and the specificity of the test for the detection of representative serotypes from all subgroups was not jeopardized (11).

Comparison of PCR product nucleotide sequences. The 140-bp nucleotide sequences obtained in this study (Ad2, Ad8, Ad10, Ad19, and Ad37) were aligned with published human adenovirus nucleotide sequences (Fig. 1). Except for Ad1 and Ad18, which showed two and three deletions, respectively, and Ad9, Ad19, and Ad37, for which only partial sequences have been published, all the sequences analyzed were 140 bp in length. Compared with the nucleotide sequence of Ad8 determined in this study, all the sequences demonstrated subgroup-specific patterns and sometimes patterns unique for a serotype.

Construction of identification scheme for adenovirus subgroups A to E. RE analysis of the nucleotide sequences of the different adenovirus serotypes demonstrated a total of five REs (*Mnl*I, *Taq*I, *Bse*RI, *Aat*II, and *Fau*I) that were found to be discriminatory, resulting in either subgenus- or sometimes subtype-specific DNA restriction patterns. Based on these patterns, an identification scheme was designed (Fig. 2). The enzyme *Mnl*I divides the analyzed adenovirus sequences into three clusters: subgroup D, subgroups A and C, and subgroups B and E.

In the first cluster (subgroup D), two patterns of restriction profiles are expected from the sequence information. The first pattern (6, 41, 43, and 50 bp) is possible with all serotypes analyzed (Ad types 8, 10, 19, 37, 17, 28 and 48), but in our experience the pattern was found only with Ad8. The second profile (6, 41, 46, and 47 bp) can be generated only with Ad types 10, 19, 37, 17, 28, and 48 but not Ad8 and is the one that we observed in practice. Further characterization of these serotypes is possible with a maximum of two REs. *Taq*I differentiates Ad8 and Ad10 from Ad types 19, 37, 17, 28, and 48, and *Bse*RI differentiates Ad8 from Ad10.

In the second cluster, *Mnl*I produces an identical restriction pattern (6 and 134 bp) with serotypes from both subgroups A and C. The two subgroups could then be easily differentiated on the basis of the restriction DNA patterns produced by *Taq*I. In the third cluster (subgroups B and E), *Mnl*I produces identical DNA restriction patterns (bands of 6, 41, and 93 bp), but *Aat*II provides distinguishable restriction profiles (it produces bands of 69 and 71 bp with subgroup E but does not cut subgroup B).

A total of 33 adenovirus clinical isolates in cell culture fluid were tested by PCR and were identified by following the scheme shown in Fig. 2. The DNA restriction profiles obtained were in complete agreement with the expected patterns.

Blind evaluation of identification scheme. There can be considerable genetic variability among adenoviruses that have the same antigenic determinants (39). A total of 56 clinical isolates of adenovirus subgroups A to E were amplified, and PCR products were blindly identified by subgenus or sometimes serotype (Fig. 3). Table 1 summarizes the results obtained and their correlation with the results of the NT test and RE analysis. Fifty-one isolates (91%) were correctly assigned to their appropriate subgroup. Those of subgroup A were identified as Ad12 or Ad31; three isolates had been typed by the NT test as type 12 and the remaining two isolates had been typed as Ad31. Among the isolates in subgroup D, based on the assumption that only Ad8, Ad10, Ad19, and Ad37 are included in the blind testing, all 16 isolates were correctly identified, including those of epidemic serotypes Ad8, Ad19, and Ad37.

Discordant results were found for five isolates (9%). Of these, two were identified as Ad10, but one had been typed as Ad7 (subgroup B) and the other had been typed as Ad9 (subgroup D) by NT, and two isolates were identified as subgroup C, but one had been typed as Ad31 (subgroup A) and the other had been typed as Ad14 (subgroup B) by NT. The last isolate had also been characterized by RE analysis of the whole genome as Ad34 or Ad35 (subgroup B). The fifth isolate was typed as Ad4a, which is in contrast to the result of Ad5 (subgroup C) by NT and that of Ad2 (subgroup C) by RE analysis.

Identification of adenovirus subgroup F. Differentiation of Ad40 and Ad41 (subgroup F) from serotypes of other subgenera in fecal specimens from patients with adenoviral gastroenteritis is of substantial clinical value. Nucleotide sequence analysis of adenovirus subgroup F (Fig. 1) revealed the possibility of including this subgroup in the identification scheme. The nucleotide sequence TGCGCA, located in the upstream primer ADRJC2 at positions 119 to 124, represents a cut site for the enzyme *Avi*II and thus would be shared by all serotypes. However, the same recognition sequence is repeated in Ad40 and Ad41 at positions 74 to 79. This cut site is not present in the analyzed sequences of the other subgroups, leading to a restriction pattern of bands of 19, 45, and 76 bp for Ad40 and Ad41 and bands of 19 and 121 bp for the serotypes from the other subgroups. In addition, Ad40 and Ad41 could be differentiated by *Taq*I, which produces a DNA restriction pattern with a band of 36 bp and two bands of 39 bp for Ad40 and bands of 36, 39, and 48 bp for Ad41. Evaluation of 8 clinical isolates of subgroup F and 12 isolates of other subgroups (A to E) by RE analysis produced patterns that agreed 100% with the expected RE patterns (Fig. 4).

DISCUSSION

Preliminary evaluation of the identification protocol described in this study involved testing of adenovirus serotypes that belong to the different subgroups. In all cases, the predicted restriction patterns were observed on gel electrophoresis. The predicted smaller fragments of 6, 9 and 11 bp could not be observed, and fragments with similar sizes (71 and 69 bp with *Aat*II for subgroup E and 41 and 43 bp or 46 and 47 bp with *Mnl*I for subgroup D) comigrated on the gel, appearing as a single band. These fragments could not be visualized or

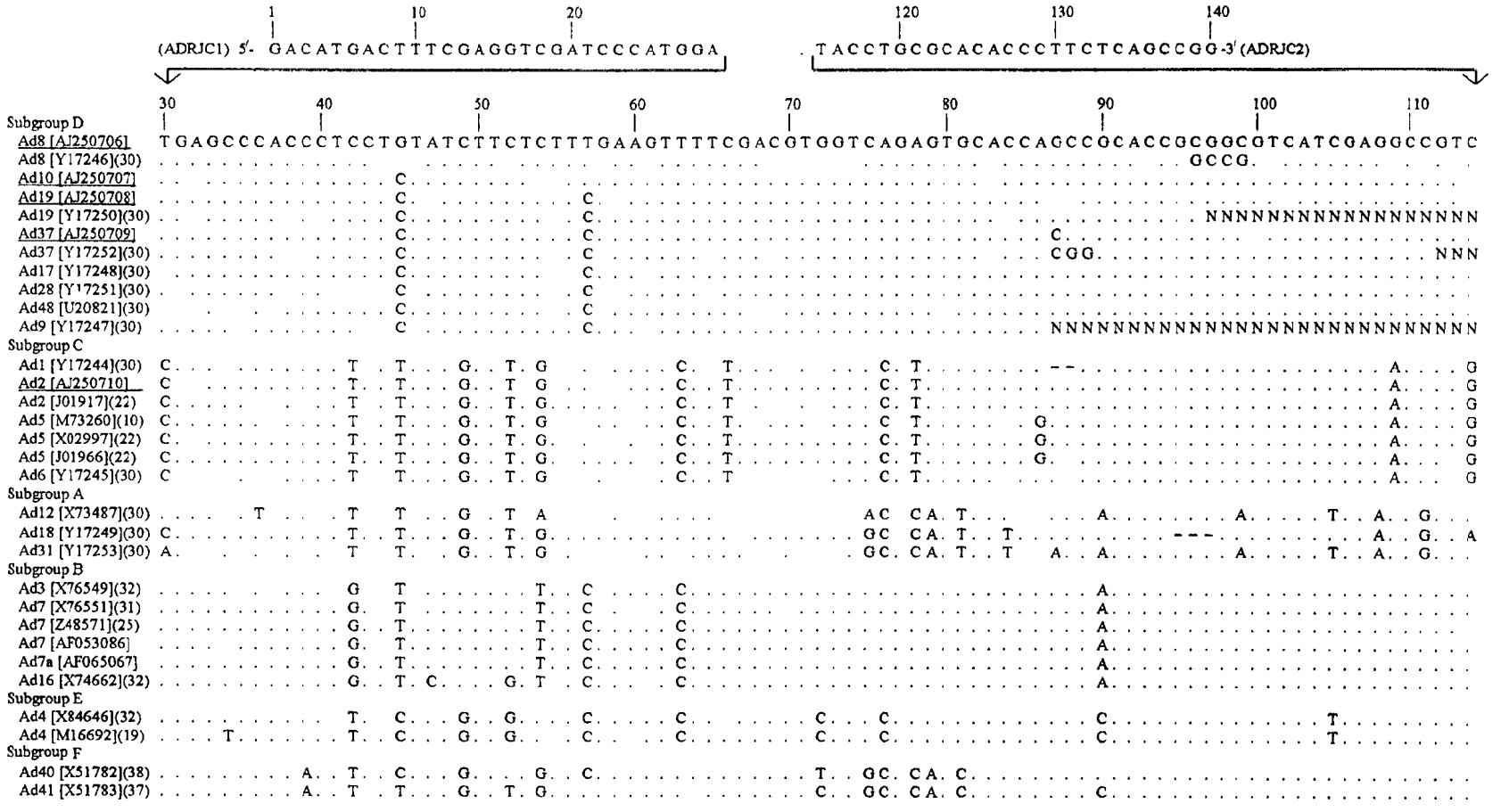

FIG. 1. Alignment of the nucleotide sequences of the 140-bp PCR products from different adenovirus serotypes. The nucleotide sequences determined in this study are underlined. The sequence accession numbers of all the serotypes are shown in brackets. The nucleotide sequence shown for ADRJC2 represents that of the complementary strand. N, undetermined sequence; −, gaps introduced for alignment of Ad1 and Ad18; periods, identical nucleotides

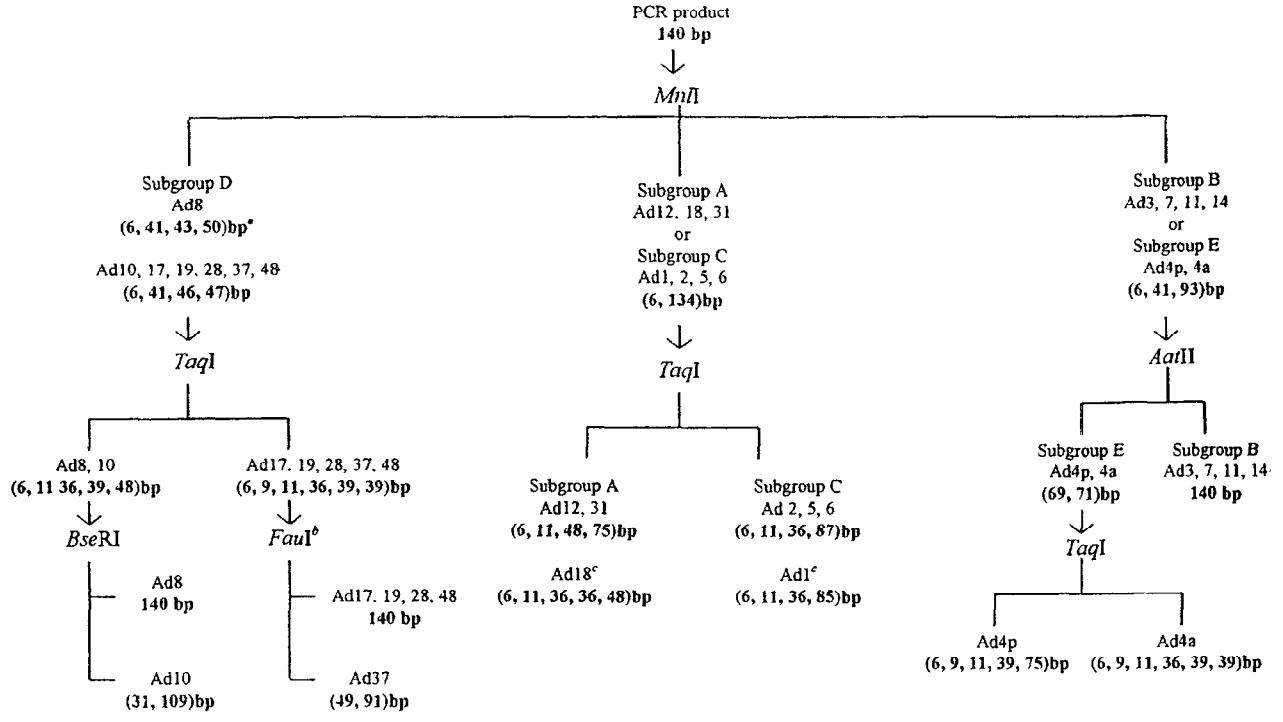

FIG. 2. Subgenus identification scheme for adenoviruses. PCR products are first treated with *MnlI* for differentiation into subgroup D, subgroups A and C, or subgroups B and E. *TaqI* discriminates between subgroups A and C, and *AatII* differentiates subgroup B from subgroup E. The enzyme *TaqI* also differentiates Ad8 and Ad10 from the remaining serotypes of subgroup D analyzed. In subgroup E, *TaqI* produces distinctive patterns for Ad4p and Ad4a. The enzyme *BseRI* cuts all the analyzed isolates of subgroup D except those of Ad8. The sizes of the DNA fragments generated are given in parentheses. [a], Ad10, Ad17, Ad19, Ad28, Ad37, and Ad48 could share this restriction pattern with Ad8 on the basis of RE sequence analysis, but in practice it does not appear to be favored. [b], based on RE sequence analysis only (*FauI* is not commercially available); [c], sequence analysis (Fig. 1) shows PCR products of 138 bp (Ad1) and 137 bp (Ad18).

separated even when a high percentage of polyacrylamide (up to 15%) was used. Smaller fragments may have been denatured into single-stranded DNA, which does not bind to ethidium bromide as efficiently as double-stranded DNA. Alternative procedures such as silver staining, which has been shown to be more sensitive than ethidium bromide in visualizing smaller fragments (9), were not applied in this study. As the small fragments were shared between the subgroups of concern and were mostly generated from cut sites located in primer ADRJC1, they would have no value in identification. In addition, exclusion of attempts to resolve these fragments led to simplified restriction patterns and shorter electrophoresis times, as visualization of smaller fragments may require a higher percentage of polyacrylamide and, thus, longer electrophoresis times for complete separation.

The accuracy and the reproducibility of the test were confirmed by blind evaluation of 56 clinical isolates that had been typed by NT and/or RE analysis of DNA extracted from infected cells. For all but five isolates the test results were in agreement with those of the NT assay (91%) [51 of 56]). This value is similar to that obtained by the study of Kidd et al. (21), in which a PCR-based identification method correlated 91.5% with the results of serotyping by NT. This discordance may be due to misidentification by NT or RE analysis, although the possibility cannot be excluded that the RE profiles of the targeted conserved regions of other adenovirus strains do not match the RE profiles demonstrated in this study and that intermediate strains may be encountered (3, 8, 17, 27).

The protocol developed in this study showed a reliable discriminatory power for adenovirus subgenera and sometimes for adenovirus serotypes and even genotypes. The important epidemic keratoconjunctivitis-causing serotypes of subgroup D (Ad8, Ad19, and Ad37), both serotypes of subgroup F (Ad40 and Ad41), and the genotypes of Ad4 (Ad4p and Ad4a) were easily differentiated, but none of the serotypes within subgroups B or C could be distinguished by this method. Nevertheless, identification of most adenoviruses to the serotype level is often no more useful to the clinician than identification to the subgenus level (21).

Although in our previous study (11) the primer pair ADRJC1-ADRJC2 failed to amplify DNA from Ad40 and Ad41, reevaluation of these primers led to successful amplification of the 140-bp PCR product from clinical isolates of Ad40 and Ad41, and analysis of the published nucleotide sequences of these serotypes revealed that they could be easily distinguished from other serotypes. Thus, for characterization of adenoviruses in fecal samples, the identification scheme could be modified to start first with the enzyme *AviII*, which places subgroup F in one cluster and the other subgroups (A to E) in another. Characterization of subgroups A to E could then follow by using the scheme in Fig. 2, and typing of Ad40 and Ad41 could be achieved with *TaqI*.

Several studies have used PCR-based identification systems for subgrouping or subtyping of adenoviruses. Kidd et al. (21) described a PCR-based subgenus identification protocol with primers which bind to regions that flank virus-associated RNA-encoding regions of the adenovirus genome, but the system does not differentiate between adenovirus serotypes of clinical importance, such as Ad8. In the study of Saitoh-Inagawa et al. (33), 14 strains from subgroups A to F were differentiated with

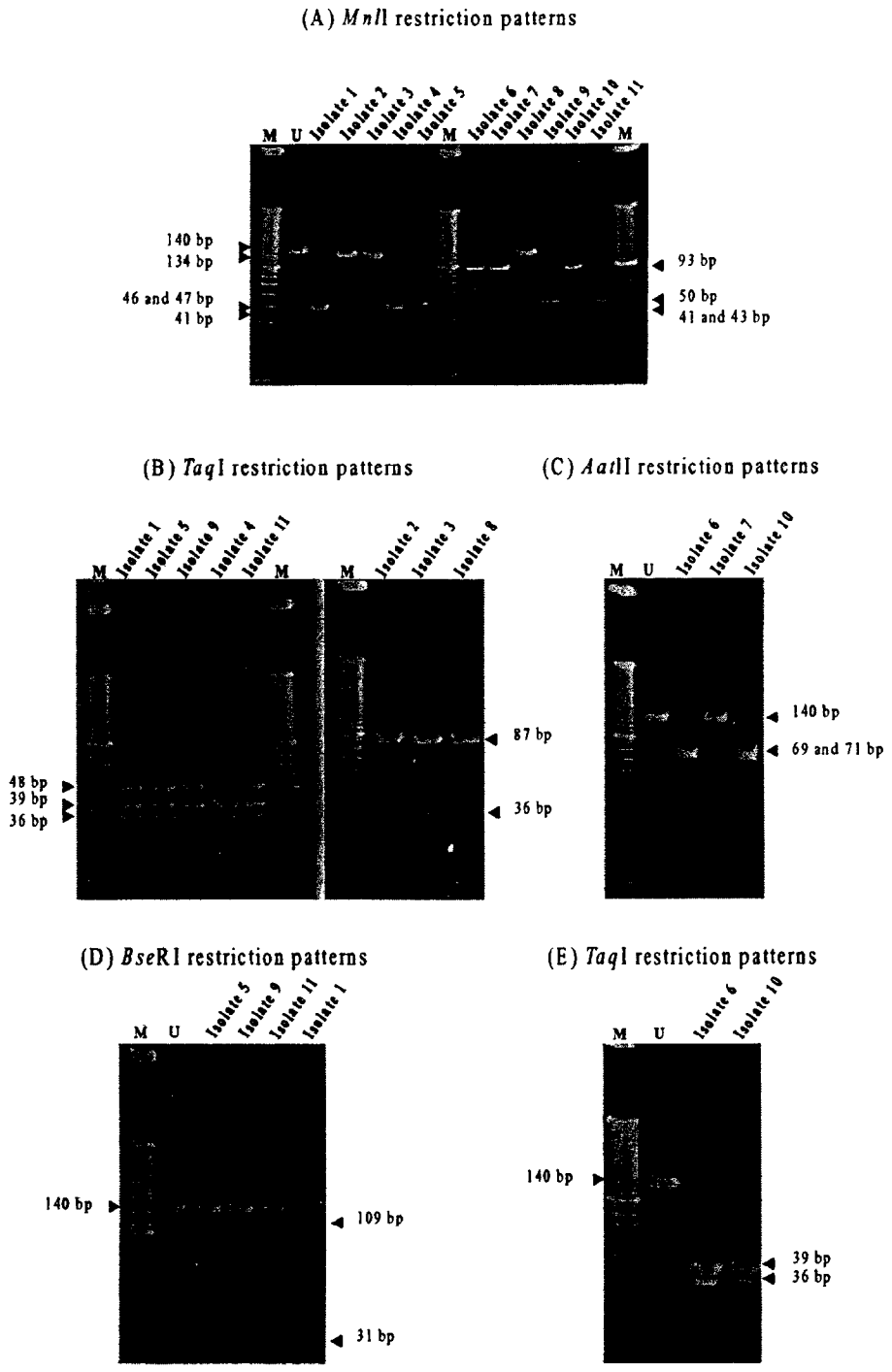

FIG. 3. Blind evaluation of 11 clinical isolates. PCR products were treated with *MnlI* (A), *TaqI* (B and E), *AatII* (C), and *BseRI* (D) by following the identification scheme and were separated on 8% polyacrylamide gels. The restriction patterns shown are consistent with Ad8 for isolates 5, 9, and 11, Ad10 for isolate 1, Ad19 or Ad37 for isolate 4, Ad4a for isolates 6 and 10, subgroup C for isolates 2, 3, and 8, and subgroup B for isolate 7. Lanes M, 10-bp DNA ladder; lanes U, uncut PCR product (140 bp).

a combination of three REs. However, the procedure requires the use of nested primers. Other approaches with PCR-based identification protocols used serotype-specific primers that target serotype-specific regions in the hypervariable or variable domains (2, 28, 29) or that rely on the sequencing of serotype-specific regions (24, 36). Whereas the former approach is prone to possible PCR failure due to variation in the genetic makeup of the target region between strains of the same serotype, the latter can be cumbersome and requires expensive instrumentation.

TABLE 1. Blind evaluation of the PCR-based identification scheme

Identity by:				No. of isolates
PCR-based identification scheme		Virus neutralization		
Subgenus	Serotype	Subgenus	Serotype	
Concordant results				
A	12 or 31	A	12	3
	12 or 31		31	2
B	NA[a]	B	3	7
			7	6
			11	2
			16	1
			21	3
C	NA	C	2	2
			5	2
D[b]	8	D	8	7
	10		10	6
	19 or 37		37	3
E	4a	E	4	7
Discordant results				
C	NA	A	31	1
		B	14[c]	1
D	10	D	9	1
	10	B	7	1
E	4a	C	5[d]	1
Total				56

[a] NA, not applicable.
[b] PCR-based identification is based on the assumption that only Ad8, Ad10, Ad19, and Ad37 are included in the blind testing.
[c] The isolate was typed as Ad34 or Ad35 by RE analysis of the whole genome.
[d] The isolate was identified as Ad2 by RE analysis of the whole genome.

The PCR-based identification method described here has its own inherent disadvantages. Although most of the REs used were chosen so that their discriminatory power is based on the appearance of different restriction fragments, one enzyme, *Bse*RI, differentiates between Ad8 and Ad10 because it cuts the latter but does not cut the former. This is also true for *Aat*II, which cuts adenoviruses of subgroup E but not those of subgroup B. In such cases, it is difficult to control whether negative results (uncut 140-bp band) indicate the expected adenovirus or merely the failure of the enzyme to cut the PCR product. For *Bse*RI, an additional confirmation is that Ad8 can be differentiated from Ad10 on the basis of RE analysis with *Mnl*I. Unfortunately, in the case of RE analysis with *Aat*II for differentiation of subgroup B and E, no other enzyme can be used to control for its activity. Incomplete cutting by the REs can also cause difficulties. This was most commonly encountered with *Bse*RI and *Mnl*I, but the problem was readily overcome by extending the incubation from 3 h to overnight.

The identification scheme used in the present study appears to be sensitive and simple. The PCR has a detection limit of 10 to 40 copies and is inclusive of all the serotypes tested in this study. The test can be applied to clinical samples, and with a maximum of four restriction enzymes, complete subgenus identification can be achieved within 24 h. For eye swab specimens, only one enzyme (*Mnl*I) is required to exclude or include adenoviruses of subgroup D. With the same enzyme it is possible to include or exclude Ad8. In addition, the test has advantages beyond adenovirus subgenus determination, which should also make it useful for epidemiological surveys. With the exception of subgenera E and F and possibly subgenus D, the subgenus identification of an adenovirus isolate can facilitate serotype identification by NT or serotype-specific PCR.

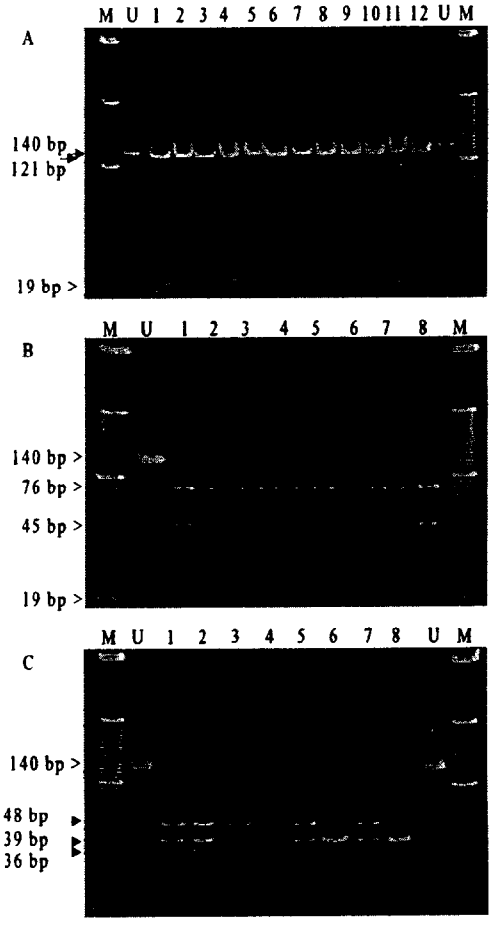

FIG. 4. Differentiation and typing of adenovirus subgroup F. PCR products from representative clinical isolates of subgroups A to F were treated with the enzyme *Avr*II (Ad types 8, 10, 2, 5, 3, 7, 11, 14, 21, 4p, 4a, and 31 [lanes 1 to 12, respectively]) (A) and Ad40 and Ad41 (B). (C) *Taq*I DNA restriction patterns for subgroup F (lanes 1 to 5 and 7, Ad41; lanes 6 and 8, Ad40). Lanes M, 10-bp DNA ladder; lanes U, uncut 140-bp PCR product.

Once the subgroup is identified, serotyping or serotype confirmation can be achieved with a minimum number of neutralizing antisera or by type-specific PCRs. The latter can be combined to include primers for all the serotypes of a subgroup (subgroup-specific multiplex PCR).

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Comparative Inactivation of Viruses by Chlorine

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The kinetics of inactivation of six enteric viruses plus simian virus 40 and Kilham rat virus by free available chlorine was studied under carefully controlled laboratory conditions. It was found that the different virus types demonstrated a wide range of susceptibility to chlorine disinfection. The rate of inactivation was greater at pH 6 than at pH 10; however, the relative susceptibilities of the different viruses were affected differently by a change in pH, suggesting that the pH influenced both the species of chlorine present and the susceptibility of the different viruses to chlorine. The presence of potassium chloride also affected the susceptibility of viruses to chlorine.

It was common practice in the early studies on the virucidal action of chlorine to compare results with those achieved with *Escherichia coli*. For example, in what was perhaps the first well-defined study, Clark and Kabler (1) found purified coxsackievirus type A2 (coxsackie A2) to be quite resistant to free available chlorine in that the amount required to inactivate the virus was between 7 and 46 times that necessary to kill *E. coli*. In a subsequent study, it was observed that adenovirus type 3 and *E. coli* demonstrated about the same degree of susceptibility to free chlorine (2).

In 1958, Weidenkopf (6) reported that 99% inactivation of poliovirus type 1 (polio 1) was achieved with 0.1 mg of free chlorine per liter in 10 min at pH 6 and 0°C. For the same degree of inactivation, increasing the pH to 7 increased the reaction time by approximately 50%. Both Weidenkopf (6) and Clark et al. (2) indicated that an increase in pH from 7 to 8.8 or 9 increased the inactivation time for viruses about sixfold. Considering the fact that the ratio of hypochlorous acid (HOCl) to hypochlorite ion (OCl^-) is a function of pH, these results indicate that HOCl is more effective in inactivating viruses than OCl^-. However, an investigation reported by Scarpino et al. (4) showed that the OCl^- ion was more effective than HOCl in inactivating polio 1. In fact, OCl^- was seven times more effective than HOCl. This observation was contrary to the findings of others (2, 6) and to the generally accepted understanding of chlorine disinfection. It was indicated by Scarpino et al. (4) that the borate-KCl-NaOH buffer used in their study may have caused the unusual HOCl-OCl^- effect.

Of the other studies on the inactivation of viruses by chlorine reported in the literature, the detailed investigation performed by Liu et al. (3) is particularly noteworthy. In discussing inactivation of viruses by chlorine, the authors point out that, of the studies performed since 1950, only about nine enteric viruses have been tested for their response to chlorine, whereas over 100 enteric viruses have been described. Considering all the available data, they concluded that there was sufficient evidence to indicate that a range of differences in resistance to chlorine existed among the viruses examined. Except for adenovirus type 3, most viruses tested have shown a resistance to chlorine about 10 times greater than that of the enteric bacteria.

With this background, Liu and his co-workers examined 20 strains of human enteric viruses for their response to free chlorine. The experiments were performed by adding individual viruses to Potomac River water which was partially treated by coagulation with alum and filtration through sand. Chlorine was added at one dosage, 0.5 mg/liter, to water having a pH of 7.8 and stored at 2°C. Samples for viral assay were withdrawn with time, corresponding to different contact times. To illustrate the wide range of resistance to chlorine of the viruses studied, the authors point out that the most susceptible was reovirus type 1, which required 2.7 min for inactivating 4 logs (99.99%) of the virus with 0.5 mg of free chlorine per liter. The most resistant was polio 2, which required 40 min for the same degree of inactivation. It would appear that this conclusion is based on extrapolating the experimental data by using first-order kinetics. On the other hand, using actual experimental data, the most resistant virus would seem to be echovirus type 12 (echo 12), which required a contact time of greater than 60 min for 99.99% inactivation. Liu et al. (3) concluded from their extrapolated

values that, "as groups, the reoviruses are definitely the least resistant to chlorine treatment; both adenoviruses and echoviruses are relatively less resistant; and the polioviruses and coxsackieviruses are the most resistant."

Because of the unusual results reported by Scarpino et al. (4) and the significance of the data presented by Liu et al. (3), coupled with the importance associated with the question of viruses in public water supplies, a research study was undertaken to confirm their observations and, at the same time, to obtain additional information on the inactivation of viruses by chlorine. Specifically, this study examined the effect of virus type, pH, and potassium chloride on the kinetics of virus inactivation by free available chlorine.

MATERIALS AND METHODS

Preparation and purification of stock viruses. Five of the picornaviruses used in this study, polio 2 (Lansing), coxsackie A9 (Griggs), coxsackie B6 (Faulkner), echo 1 (Farouk), and echo 5 (Noyce), were obtained from the National Institutes of Health, Bethesda, Md. The sixth picornavirus, polio 1 (Mahoney), was provided by Gerald Berg, U.S. Environmental Protection Agency, Cincinnati, Ohio. All six of these viruses were cultured on Buffalo green monkey kidney cells; virus titer assays were determined with the same cell line by the plaque technique.

Simian virus 40 (SV40), a papovavirus, was obtained from Lowell Hager, Department of Biochemistry, University of Illinois. This virus was cultured on CV-1 cells, a derivative of the African green monkey kidney cell line. Virus titers were obtained by the plaque technique.

Kilham rat virus, a parvovirus, and the rat nephroma cells in which the virus was cultured were provided by Lois A. Salzman, National Institutes of Health. The plaque assay technique was not practical with Kilham rat virus, since the virus will proliferate only in actively growing cells. Therefore, the virus was assayed by the hemagglutination (HA) technique, by plating quintuplicate portions of a virus sample which had been exposed to chlorine on rat nephroma cells. After the appearance of cytopathic effect, the cells containing progeny virus were scraped from the plates, frozen and thawed three times, and centrifuged, and the HA technique was used on the supernatant. Data for each time point were recorded as the average of five separate HA titers.

All virus preparations were plaque purified and serially passaged until stocks of adequate titers were obtained, i.e., 10^6 to 10^9 plaque-forming units/ml. The plaque-purified stocks were processed to reduce the chlorine demand of the preparations, using the procedure described by Sharp et al. (5); equal portions of pooled virus fractions were stored at −70°C.

Preparation of CDF buffer and chlorine solution. Chlorine demand-free (CDF) buffer was used as the suspending medium in all of the inactivation experiments. The CDF buffer was prepared by adding a sufficient amount of Clorox (Clorox Corp., Oakland, Calif.) to deionized distilled water to achieve a free chlorine residual of approximately 5.0 mg/liter. After storage at room temperature for several days, this chlorinated water was used to prepare the appropriate buffer. For experiments performed at pH 6 and 7.8, a 0.05 M sodium phosphate buffer was used. A 0.05 M borate buffer (H_3BO_3-NaOH) without KCl was used with the experiments performed at pH 10. After addition of chemicals to the chlorinated water, both the phosphate and borate buffers were boiled for several minutes, cooled to room temperature, and exposed to ultraviolet light for 48 to 72 h to achieve dechlorination. All buffer solutions were analyzed for the absence of chlorine by the orthotolidine test before use. When the orthotolidine test was negative, the buffers were considered to be chlorine demand-free.

The stock chlorine solutions (0.1% available chlorine) used in the inactivation experiments were prepared by using Clorox, since these solutions proved to be more stable than those prepared from chlorine gas. To compare the two chlorine solutions (Clorox versus chlorine gas), an inactivation experiment was performed with polio 1 by using both solutions; no difference was observed in the inactivation rate with the two chlorine solutions.

Experimental equipment and procedure. The virus inactivation experiments were performed by using a multiple laboratory stirrer (Phipps and Bird, Richmond, Va.), with separate stainless-steel beakers containing CDF buffer being used to monitor simultaneously virus inactivation, temperature, and free available residual chlorine (Wallace and Tiernan, Amperometric Titrator, Penwalt Corp., Belleville, N.J.). To initiate a virus inactivation experiment, 1 ml of a virus preparation was added to 400 ml of CDF buffer having the appropriate free available residual chlorine concentration. At various times afterward, 5-ml portions were removed and added to sodium thiosulfate (12 mg/ml) to neutralize the chlorine, and the remaining viruses were titered.

The results present data from at least three separate, identical inactivation experiments. Experimental error was generally less than 20%. The 99% inactivation data were obtained graphically by plotting percentage of virus survival against time of exposure to chlorine.

RESULTS

Effect of pH on chlorine inactivation. Experiments were performed with polio 1 and 2, echo 1 and 5, and coxsackie A9 and B5. These viruses were selected for their reported wide range of susceptibility to chlorine (3). Of the above viruses, polio 2, echo 1, and coxsackie B5 were identified as the most resistant to chlorine of the respective virus pairs. To evaluate the effect of pH and the resulting predominant species of chlorine on virus inactivations, the inactivation of these six viruses was studied at pH 6 and 10. Chlorine inactivation studies were also performed at pH 7.8 with echo 1 and 5, coxsackie

B5, and polio 1 in order to have data comparable to those of Liu et al. (3). Table 1 summarizes the results, giving the time required for 99% inactivation of the six picornaviruses at pH 6 and 7.8 in phosphate buffer and at pH 10 in borate buffer.

All of the chlorine inactivation experiments were performed at 5 ± 0.2°C. The pH never varied more than 0.5 unit during any experiment. The initial virus titer in each experiment was about 10^4 plaque-forming units/ml, and the dosage of chlorine was 0.50 to 0.53 mg/liter. The residual chlorine level, as measured amperometrically at the end of each experiment, often decreased but generally not more than 0.03 mg/liter. This decrease represents approximately a 6% chlorine demand. Table 1 gives the range of residual chlorine at the end of each of three separate experiments for each of the viruses.

There was a significant difference in the time required for 99% inactivation of the various viruses at pH 6 and 10 (Table 1). In every case, the rate of inactivation at pH 10 was dramatically less than at pH 6. For example, echo 1 was 99% inactivated in 0.5 min at pH 6 and in 96 min at pH 10, whereas polio 1 was 99% inactivated in 2.1 min at pH 6 and 21 min at pH 10.

The rank ordering in Table 1 demonstrates the wide range of susceptibilities of related viruses to chlorine disinfection. For example, at pH 10, coxsackie B5 was 40 times more resistant than coxsackie A9. Interestingly, there were several cases in which the relative susceptibility to chlorine was altered (rank ordering) between pH 6 and 10, suggesting important effects of pH on the virion as well as on the chlorine species. Echo 1 was the second most susceptible virus at pH 6 but was the most resistant at pH 10. Polio 1 was one of the most resistant viruses at pH 6; however, it was relatively susceptible at pH 10 when compared with the other viruses at this pH value. This can be seen most clearly by inspection of Table 2, in which the time required for 99% inactivation of the viruses and ratios of inactivation rates at pH 6 and 10 are compared. It should be noted that at pH 6 and 0°C, approximately 98% of the chlorine was in the hypochlorous acid (HOCl) form, whereas at pH 10 and 0°C, only 0.5% of the chlorine was present as HOCl, the remainder being present in the dissociated form as hypochlorite ion (OCl^-). Even at pH 7.8, differences in relative susceptibility appeared when rank ordered and compared with results at pH 6 (Table 1). Polio 1 and echo 1 were inactivated at approximately the same rate at pH 7.8, whereas their rates were quite different at pH 6. Of particular interest was the observation that polio 1 was inactivated more rapidly at pH 7.8 than at pH 6. This suggests that the effect of pH in this range (pH 6 to 7.8) may be greater on the structure and reactivity of the virus than on the species of chlorine.

Figure 1 shows the differences in the kinetics of inactivation of the two types of polio- and coxsackieviruses at pH 6 and 10, with 0.5 mg of free available chorine per liter and at 5°C. Each pair of viruses from the same subgroup is shown on one figure for purposes of comparison, and each curve represents the average of three or more separate experiments. It is significant to note that some virus types closely resembled each other in their kinetic response to chlorine,

TABLE 1. *Time required for 99% inactivation by free residual chlorine at 5.0 ± 0.2°C*

pH	Concn of free chlorine[a] (mg/liter)	Virus type	Time for 99% inactivation (min)	Rank ordering
6.00	0.46–0.49	Coxsackie A9	0.3	1
6.00	0.48–0.49	Echo 1	0.5	2
6.00–6.02	0.48–0.51	Polio 2	1.2	3
6.00–6.03	0.38–0.49	Echo 5	1.3	4
6.00	0.47–0.49	Polio 1	2.1	5
6.00–6.06	0.51–0.52	Coxsackie B5	3.4	6
		Coxsackie A9	ND[b]	
7.81–7.82	0.47–0.49	Echo 1	1.2	1
		Polio 2	ND	
7.79–7.83	0.48–0.52	Echo 5	1.8	3
7.80–7.84	0.46–0.51	Polio 1	1.3	2
7.81–7.82	0.48–0.50	Coxsackie B5	4.5	4
10.00–10.01	0.48–0.50	Coxsackie A9	1.5	1
10.00–10.40	0.49–0.51	Echo 1	96.0	6
9.89–10.03	0.48–0.50	Polio 2	64.0	4
9.97–10.02	0.49–0.51	Echo 5	27.0	3
9.99–10.40	0.50–0.52	Polio 1	21.0	2
9.93–10.05	0.50–0.51	Coxsackie B5	66.0	5

[a] Range of measured free chlorine residual in the test reactor at the termination of each of three separate experiments.

[b] ND, Not determined.

TABLE 2. *Comparison of virus inactivation by free residual chlorine at pH 6.0 and 10.0 and 5.0 ± 0.2°C*

Virus type	Time for 99% inactivation (min)		
	pH 6.0	pH 10.0	Ratio[a]
Coxsackie A9	0.3	1.5	5
Echo 1	0.5	96.0	192
Polio 2	1.2	64.0	53
Echo 5	1.3	27.0	21
Polio 1	2.1	21.0	10
Coxsackie B5	3.4	66.0	19

[a] Time required at pH 10.0/time required at pH 6.0.

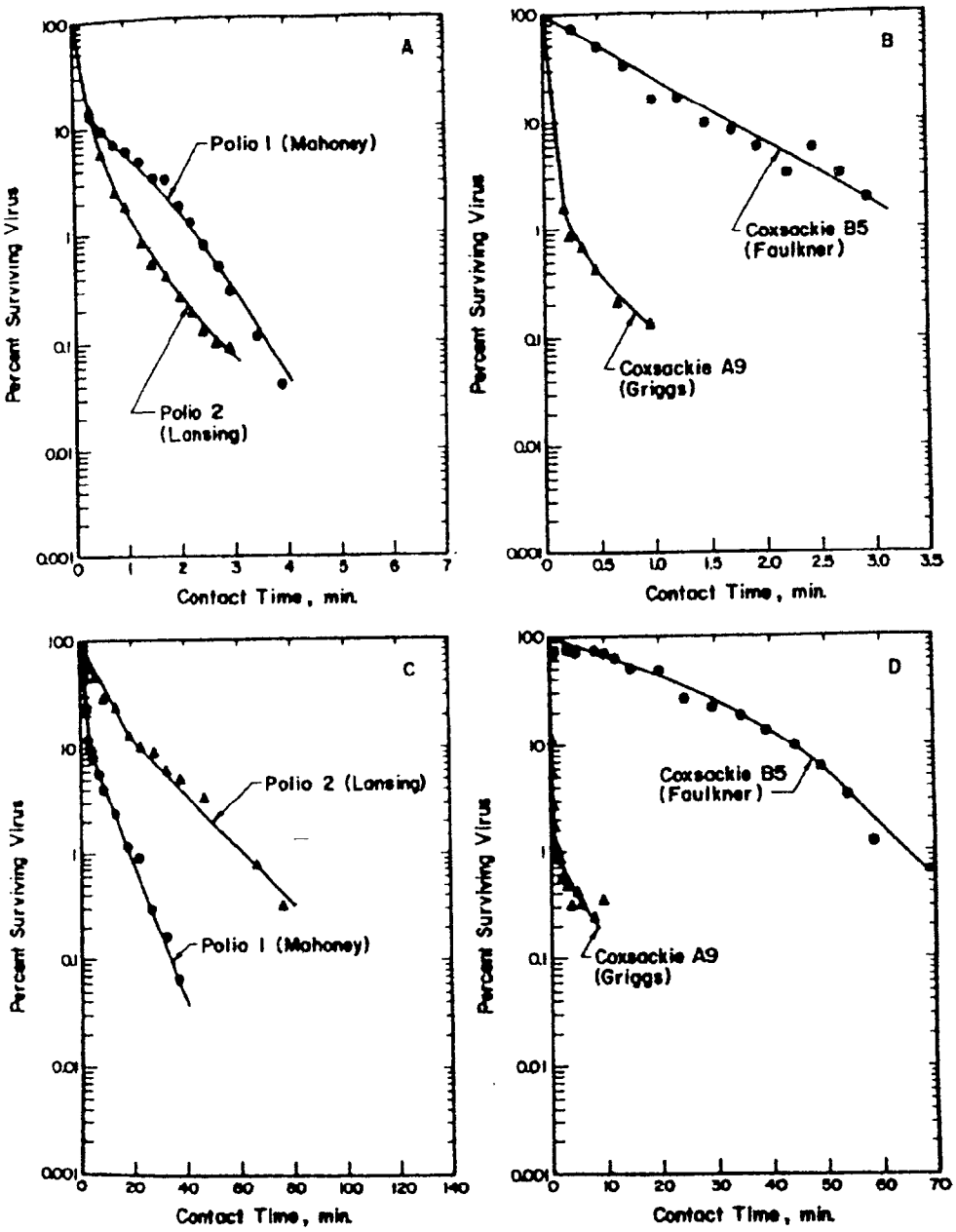

FIG. 1. *Inactivation kinetics at 5°C. (A) Polio 1 and polio 2 with 0.47 to 0.51 mg of free available chlorine per liter at pH 6.0; (B) coxsackie A9 and coxsackie B5 with 0.46 to 0.52 mg of free available chlorine per liter at pH 6.0; (C) polio 1 and polio 2 with 0.48 to 0.52 mg of free available chlorine per liter at pH 10.0; (D) coxsackie A9 and coxsackie B5 with 0.48 to 0.51 mg of free available chlorine per liter at pH 10.0.*

whereas others did not. For example, coxsackie A9 at pH 6 showed a rapid decrease in titer followed by a somewhat slower rate of inactivation, whereas coxsackie B5 appeared to be dramatically more resistant to chlorine (Fig. 1B). The half-life of coxsackie A9 was about 2 s, whereas the half-life of coxsackie B5 was approximately 0.5 min, a 15-fold difference.

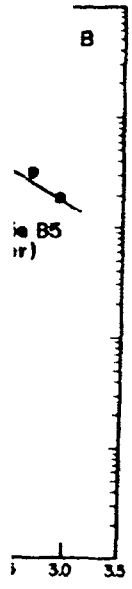

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Figure 1A shows the kinetics of inactivation of polio 1 and 2 at pH 6 and 5°C with 0.5 mg of free available chlorine residual per liter. These viruses had similar inactivation kinetics, contrary to the results of the two coxsackieviruses (Fig. 1B). Initially, both polioviruses had a half-life of approximately 8 to 10 s. However, a significant difference in the rate of inactivation for polio 1 and polio 2 appeared after approximately 30 s of exposure to chlorine. Two logs of polio 1 were inactivated after 2 min, whereas polio 2 was inactivated to the same extent in half the time. This is a consequence of the nonlinearity of the inactivation curves.

This nonlinearity was most likely due to a difference in degree of aggregation between the two viruses, although differences in the rate at which chlorine and the neutralizing thiosulfate solution penetrate the capsid could also have contributed. Limited results with sucrose gradients suggested that virus aggregation amounted to 0.5 to 8% of the particles in the virus stock preparations (data not shown). This is consistent with the deviation from single-hit kinetics of poliovirus, between 1- and 2-log inactivation (see Fig. 1A). Whether strain-specific variations in the degree of aggregation consistently generate differences in inactivation kinetics remains to be determined. The results with polio 1 quantitatively agree with those reported by Weidenkopf (6), including the biphasic nature of the curve.

Since phosphate buffer was used in preparing the CDF buffer solution at pH 6 and borate at pH 10, it was important to determine whether the buffers themselves affected the rates of inactivation. Figure 2 compares the kinetics of chlorine inactivation of polio 1 at pH 7.8, using both the borate and phosphate CDF buffer solutions. The curves are indistinguishable, indicating that the buffer ions were probably not responsible for the previous results.

Chlorine inactivation experiments were performed at pH 7.8 with echo 1, coxsackie B5, and echo 5 in order to have data comparable with those of Liu et al. (3). Figure 3 compares the chlorine inactivation of these three viruses at pH 7.8 with 0.5 mg of free available chlorine per liter and at 5 ± 0.2°C. The times for 99.99% inactivation of these three viruses were less than the times reported by Liu et al. (3) in their Potomac River water study. Liu et al. (3) found, assuming a first-order reaction and by extrapolating their experimental data, that echo 5 required 8 min, polio 1 required 16.2 min, echo 1 required 27 min, and coxsackie B5 required 39.5 min to achieve 99.99% inactivation with 0.5 mg of free available chlorine per liter in Potomac River water at pH 7.8 and 2°C. Extrapolation of

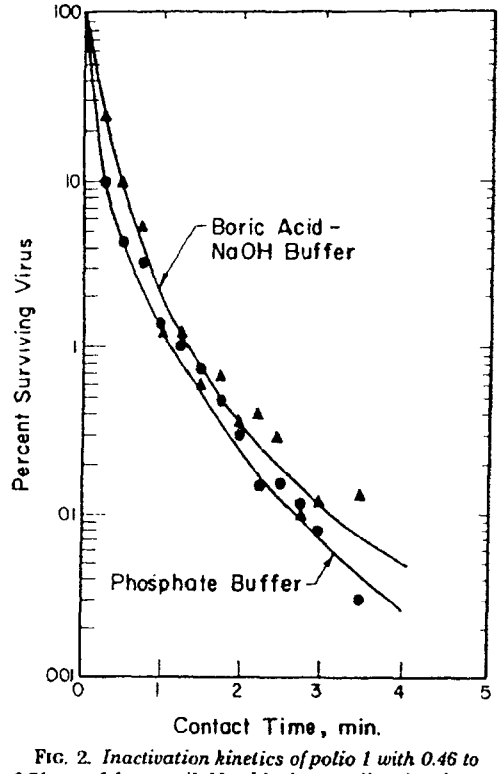

FIG. 2. *Inactivation kinetics of polio 1 with 0.46 to 0.51 mg of free available chlorine per liter in phosphate buffer and boric acid-NaOH buffer at pH 7.8 and 5.0°C*

the data generated in this study indicated that echo 5 required 4.5 min, polio 1 required 3 min, echo 1 required 4.7 min, and coxsackie B5 required 6.7 min for the same degree of inactivation of the virus with 0.5 mg of free available chorine per liter at pH 7.8 but with CDF buffer solution and at 5°C. Comparing the results of these two independent studies and neglecting the small difference in temperature between the two sets of data, it would appear that the inactivation of the virus in natural water, i.e., Potomac River water, may differ significantly from that which occurs in CDF buffer solution. It is entirely possible that other technical differences could account for the divergence in results obtained in this study and those of Liu et al. (3).

Chlorine inactivation as a function of virus group. The picornaviruses, having ribonucleic acid as their genetic material, appeared to differ widely in their susceptibility to chlorine (Table 1). The possibility that virus groups other than the enteroviruses might also differ greatly with respect to chlorine susceptibility was also considered. Thus, the susceptibility to chlorine

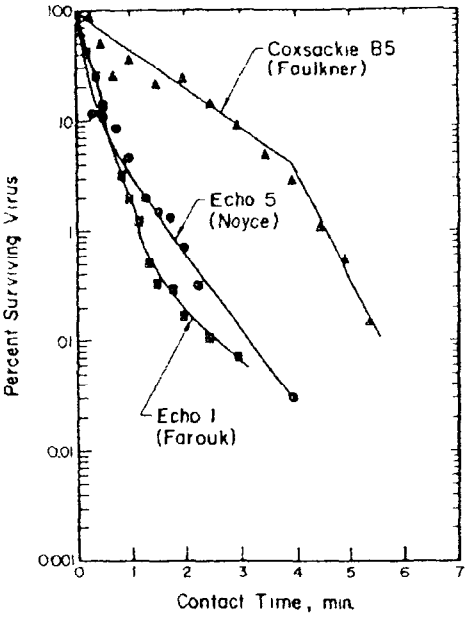

FIG. 3. *Inactivation kinetics of echo 1, echo 5, and coxsackie B5 with 0.47 to 0.52 mg of free available chlorine per liter at pH 7.8 and 5.0°C*

of Kilham rat virus, a parvovirus containing single-stranded deoxyribonucleic acid, and SV40, a double-stranded deoxyribonucleic acid oncogenic virus, was determined. Although neither virus has been detected in water supplies, the response of both viruses to chlorine does confirm the observation that there is a wide range of chlorine susceptibility among the viruses. Tables 3 and 4 give the kinetics of inactivation of Kilham rat virus and SV40, respectively, at pH 6 with 0.5 mg of free available chlorine per liter and at a temperature of 5°C.

Kilham rat virus was no more resistant to chlorine than any of the picornaviruses, being 99% inactivated in less than 20 s. SV40 was even more susceptible to chlorine than Kilham rat virus, being 99% inactivated in 10 s. Even though SV40 is considered by tumor virologists to be very hardy under adverse conditions, it appears to be extremely susceptible to chlorine.

Effect of potassium chloride on chlorine inactivation. Experiments were performed at 5°C with 0.5 mg of free available chlorine per liter, using 0.05 M phosphate buffer (pH 6) and boric acid-NaOH buffer (pH 10) with and without 0.05 M KCl added, to further investigate the reversal effect of KCl that was observed by Scarpino et al. (4). Their results indicated that inactivation of polio 1 was more effective at pH levels where the free available chlorine was predominantly in the form of OCl^- rather than in the form of HOCl. It was reported that inactivation of polio 1 at pH 10 was seven times more rapid than at pH 6. This is contrary to the findings of other investigators. The buffer used by Scarpino et al. (4) in their pH 10 experiments was 0.05 M borate buffer (H_3BO_3-KCl-NaOH). The pH 10 buffer system used in this study was also boric acid and sodium hydroxide, but with and without added KCl.

The time required for 99% inactivation of polio 1 by 0.5 mg of free available chlorine per liter at pH 10 was decreased approximately 50 times by the addition of 0.05 M KCl to the boric acid-NaOH buffer system (Fig. 4). The rate of inactivation of polio 1 in 0.05 M phosphate buffer at pH 6, with and without 0.05 M KCl, was also studied (Fig. 5). Once again, the addition of KCl decreased sixfold the time for 99% inactivation of polio 1.

DISCUSSION

The experimental results reported from this study demonstrate the importance of three variables affecting the susceptibility of viruses to chlorine disinfection: the virus type, the pH, and the ionic nature of the suspending medium. That different viruses can display large differences in susceptibility to chlorine was obvious even within the picornavirus group, which displayed a 64-fold range in the time required for 2-log inactivation at pH 10. A more extensive survey of virus types could revel even wider differences, as indicated by the fact that SV40 was even more susceptible to chlorine at pH 6 than was

TABLE 3. *Chlorine inactivation of Kilham rat virus by free residual chlorine at 5.0 ± 0.2°C*

Expt no.	pH	Concn of free chlorine in test reactor (mg/liter)	HA titer				
			Control	10 s	20 s	30 s	60 s
1	6.01	0.48	1:1,638	1:1,792	1:3.2	1:1.6	0
			(100)[a]	(109)	(0.19)	(0.09)	0
2	6.04	0.49	1:563	1:243	1:3.2	0	0
			(100)	(43)	(0.56)	0	0

[a] Percentage survival is given in parentheses.

TABLE 4. *Chlorine inactivation of SV40 by free residual chlorine at 5.0 ± 0.2°C*

Expt no.	pH	Concn of free chlorine in test reactor (mg/liter)	% of virus surviving			
			Control	10 s	20 s	30 s
1	6.01	0.49	100	1.13	0	0
2	6.02	0.48	100	1.67	0.02	0
3	6.02	0.48	100	0.69	0	0

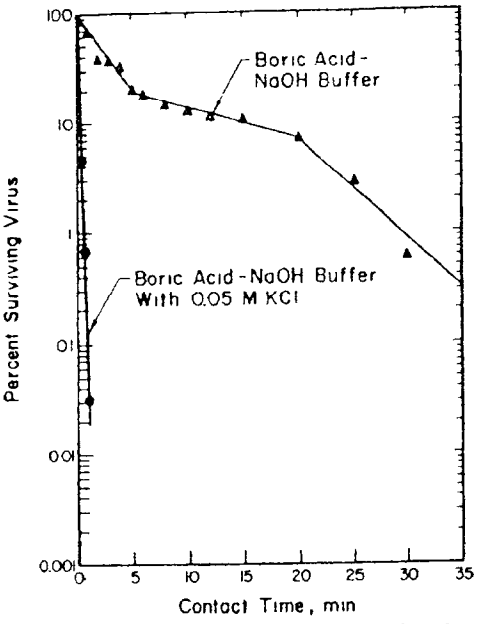

FIG. 4. *Inactivation kinetics of polio 1 with 0.46 to 0.49 mg of free available chlorine per liter in 0.05 M boric acid-NaOH buffer, with and without 0.05 M KCl, at pH 10.0 and 5.0°C.*

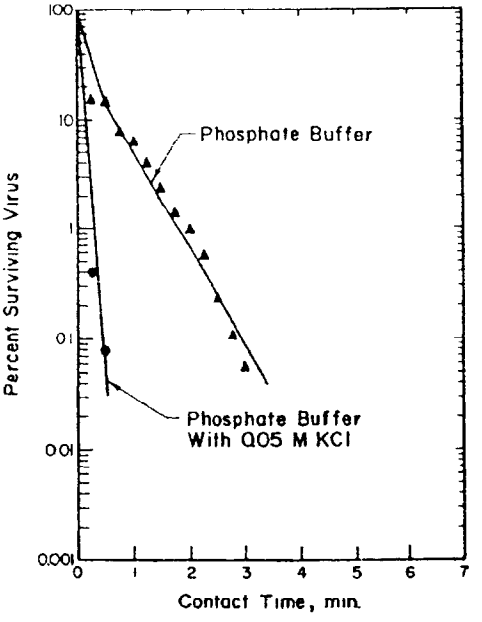

FIG. 5. *Inactivation kinetics of polio 1 with 0.48 to 0.49 mg of free available chlorine per liter in 0.05 M phosphate buffer, with and without 0.05 M KCl, at pH 6.0 and 5.0°C.*

the most susceptible of the picornaviruses tested. Liu et al. (3) have previously reported substantial differences in chlorine susceptibility between different picornavirus types. However, the results reported here in general reveal a greater degree of chlorine susceptibility than was observed by Liu et al. (3). This discrepancy may be a consequence of the use of Potomac River water by Liu et al. (3), whereas CDF buffer prepared from distilled water was used in the current study. It should be noted that much of the data presented by Liu et al. (3) were based on extrapolation from a few early time points, assuming first-order kinetics. This assumption may not be warranted since many of the viruses do not display first-order kinetics of inactivation over the entire range, presumably due in part to the aggregation of the virus.

Since HOCl is more reactive than OCl^-, it was not surprising to find that the viruses examined were inactivated more rapidly by chlorine at pH 6 than at pH 10. However, the relative susceptibility of the different viruses changed dramatically with pH. Thus, echo 1, which was the second most susceptible picornavirus at pH 6, was the most resistant at pH 10. Increasing the pH from 6 to 7.8 reduced the proportion of HOCl from approximately 98 to 50%, but had only a slight effect on the rate of inactivation of most of the viruses studied. This indicates that pH affects not only the predominant species of chlorine, but perhaps the susceptibility of the virion to inactivation also. The suggestion that hydrogen ion can alter the reactivity of virions with chlorine is supported by the observation that inactivation of polio 1 was dramatically affected by the presence of potassium chloride, being accelerated 50-fold at pH 10 and only 6-fold at pH 6.

The wide range of susceptibility to chlorine disinfection displayed by different viruses, the large effects on inactivation due to pH and ions, and the fact that the effects of pH and ions are themselves affected strongly by the virus type make it highly unlikely that a single virus will prove to be a suitable indicator for determining the adequacy of the disinfection process in treating a water supply.

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a number of other s[illegible].[8-12,16] Among neutropenic patients, however, *monas* was found to be a pathogen. Among G[illegible]positive organisms the relative incidence of coagulase-positive *Staphylococcus* is somewhat higher in this study than in other recent reports. Because patients with exit site infections were more likely to have *S. aureus* isolated from the blood than coagulase-negative *Staphylococcus* (63 *vs.* 25%), this predominance of *S. aureus* may reflect the large number of local infections in this population.

These findings suggest that the presence of an indwelling catheter, by breeching the epidermal barrier, increases the risk of sepsis in all patients but that the added presence of neutropenia may alter the spectrum of pathogens responsible for infection. Furthermore in children with Broviac-type catheters the presence of an exit site infection and fever is highly predictive of bacteremia and should be initially managed as such.

Because of the relatively small number of patients studied, individual factors such as age, duration of catheter use, type of indwelling catheter (Portacath® *vs.* Broviac) or underlying malignancy which may serve to identify those patients at especially high or low risk of bacteremia cannot be identified. This study sample, however, is broadly representative of the population of oncology patients at a pediatric referral center. We therefore conclude that immediate empiric antibiotic therapy is warranted in all pediatric cancer patients with fever and an indwelling central venous catheter, regardless of their absolute neutrophil count. Initial choice of antibiotic coverage should include agents against a broad range of pathogens, including coagulase-negative staphylococci and other common epidermal organisms.

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Astroviruses as a cause of nosocomial outbreaks of infant diarrhea

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During a 16-month study period at a children's hospital, 32 children developed nosocomial gastroenteritis caused by astroviruses. Twenty-five of these occurred during 2 epidemic outbreaks in medical and surgical infants' wards. From the community, 13 confirmed cases were admitted during the study period. Both community-acquired and nosocomial cases occurred during autumn, winter and early spring. The attack rates during outbreaks ranged between 7 and 62% and were highest among children with underlying gastrointestinal diseases. Diarrhea and vomiting were the most common clinical manifestations. The median duration of symptoms was 4 days and that of virus excretion was 5 days.

Hospital infection with astroviruses is common and usually affects children less than 2 years of age. The probable mode of transmission is spread via contaminated hands.

INTRODUCTION

Nosocomial viral gastroenteritis is common in infant wards.[1–4] Several studies have described the principal role of rotavirus[1,5,6] although Norwalk-like virus and astrovirus are also common.[2,7]

Astroviruses are small round particles, 28 to 30 μm in diameter, with a five- or six-pointed star configuration. It is a nonenveloped RNA-containing virus with five distinct polypeptides.[8] It was first described in humans by Appleton and Higgins[9] and Madeley and Cosgrove[10] in infants with mild diarrhea and vomiting. Strains of astrovirus have been associated with outbreaks of diarrhea in lambs[11] and of hemorrhagic hepatitis in ducklings.[12] Five human serotypes have been described.[13]

The fewer studies on astrovirus compared with those on rotavirus could probably be explained by the milder symptoms of astrovirus infections and by the difficulties in identifying and isolating the virus.[14,15] As a result we assessed the role of astrovirus as an etiologic agent of nosocomial and community-acquired gastroenteritis and investigated the clinical features, epidemiology and pattern of nosocomial spread in two outbreaks.

MATERIALS AND METHODS

Hospital and wards. St. Göran's Children's Hospital is a 250-bed pediatric teaching hospital with pediatric surgery and intensive care. It serves as a tertiary referral center. The hospital also has its own catchment area, serving a population of 1 million people of various social and ethnic backgrounds.

Isolation facilities are mainly provided by an infectious disease unit of 15 rooms. Patients with diarrhea are, whenever possible, admitted to this unit. The pediatric and surgical wards caring for children younger than the age of 2 years are situated on the same hospital floor. Each ward has 10 rooms, including 2 single rooms that can be used for isolation purposes. The patients are cared for by unshared nursing staff but physicians and medical students move between wards.

Definitions. Diarrhea was defined as an increase in frequency to more than two per 24 hours and/or a change in consistency of stool. Gastroenteritis was defined as nosocomial when onset of diarrhea and/or vomiting began at least 72 hours after admission or less than 72 hours after discharge. Study patients were not routinely followed, but in one case readmittance became necessary.

The attack rate was defined as the number of infected patients, divided by the number of patients at risk (those hospitalized for more than 3 days)

Data collection. This is a retrospective study covering 16 months from September, 1987, to December, 1988. This period included two hospital outbreaks of astrovirus gastroenteritis. Stools from all cases of

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nosocomial diarrhea, as identified by the criteria above, were routinely sent for virus detection. However, the majority of community-acquired cases of gastroenteritis of short duration admitted to the infectious disease ward were not routinely studied. Because of rapid electron microscopic techniques (see below) we became aware of the two clusters of astrovirus infection when they occurred. During those periods stool samples were also taken from asymptomatic roommates and family members and, during the first outbreak, among staff. Serologic studies were not routinely performed. Fecal specimens were sent for bacteriologic examination according to the clinical judgment of the attending physician.

Forty-eight follow-up samples were obtained from 17 infected children, including 2 asymptomatic. More than 1 follow-up sample were obtained in 9 children (Fig. 2).

The medical records of the patients with astrovirus-positive stools were examined. Data on sex, age of onset, underlying disease, nutritional status, clinical symptoms and other concomitant virus were recorded.

Virological methods. Routine diagnostic service included electron microscopy of stool samples from all children younger than 7 years of age attending St. Göran's Children's Hospital. Stool specimens sent to the virologic laboratory were examined by negative contrast electron microscopy. One drop of a 10% suspension of feces was placed on a Formvar carbon-coated copper grid for 1 minute. The specimen was negatively stained with 2% phosphotungstic acid (pH 6.0) and air dried. Two grids from each specimen were examined in a JEOL JEM-100C electron microscope at ×40 000. The specimen was considered negative if no virus was detected after 15 minutes of microscopic examination.

Virus isolation was performed on all cases in which no agent was identified by electron microscopy, primarily to detect nonenteric adenoviruses and enteroviruses. For this procedure 0.2 ml of the 10% fecal suspension was inoculated into tissue culture tubes of HeLa cells, green monkey kidney cells (GMK-AM) and human rhabdomyosarcoma (Rd) cells. The cells were observed every 3 to 4 days for 2 weeks.

Statistical method. Statistical analysis was performed by the chi square and Fisher exact tests, and $P < 0.05$ was considered significant.

RESULTS

Incidence and epidemic outbreak. We found 78 episodes of nosocomial diarrhea with a defined etiology in 68 patients. In 7 episodes more than 1 virus was found; 43% were caused by rotavirus and 44% by astrovirus. Thirty-two of the 45 astrovirus infections were nosocomial whereas 134 of 167 rotavirus infections diagnosed during the same period were community-acquired ($P < 0.001$) (Table 1).

There were two outbreaks in the last 2 weeks of September, 1987 (11 cases), and October-November, 1988 (15 cases) (Fig. 1). Each outbreak could have been related to one community-acquired index case. Spread between the infant medical and surgical wards probably occurred by transfer of patients before symptoms of gastroenteritis had appeared (Fig. 1). Altogether 26 episodes of gastroenteritis occurred in 26 children during these 2 outbreaks. The mean interval between index case and secondary episodes was 3 days (range, 2 to 13 days). The cases of nosocomial diarrhea in the infectious disease ward could not be traced to a known contact.

The median duration of hospitalization was 8 weeks (range, 1 to 46 weeks) and the median interval between admission and onset was 10 days (range, 3 to 120 days). The attack rate in the medical ward during the first outbreak was 23% whereas the attack rate in the surgical ward was 62%. During the second outbreak the attack rates were 7 and 13%, respectively. The cases of nosocomial astrovirus gastroenteritis outside the epidemic outbreaks were distributed between all wards and apparently did not give rise to secondary cases.

During the first outbreak fecal samples were obtained from 27 staff members, 10 roommates, 3 parents and 3 siblings. One parent and 2 roommates (Fig. 2, Patients 6 and 7) were positive but asymptomatic. Two siblings, cared for at home, were positive and symptomatic. All staff members were negative.

Characteristics of children with nosocomial and community-acquired astrovirus gastroenteritis. The male:female ratio was 12:20 among nosocomial and 6:7 among community-acquired cases. The distributions in the age groups <1, 1 to 2, and >2 years were 28, 4, 0 and 6, 5, 2, respectively ($P < 0.01$). The age distribution of the infants with nosocomial gastroenteritis did not differ from that of other patients in the wards. Underlying diseases are shown in Table 2.

Sixteen of 32 cases of nosocomial astrovirus gastroenteritis were associated with gastrointestinal diseases (mainly gastroesophageal reflux and malformations in the surgical ward, failure to thrive and celiac disease in the medical ward). During the two cluster periods the numbers of children with gastrointestinal diseases *vs.* all children were 25 of 181 (medical ward) and 50 of 192 (surgical ward). Thus patients with gastrointestinal disease were overrepresented among infected children ($P < 0.001$ by chi square test).

Among the children with community-acquired astrovirus gastroenteritis, one was admitted with trauma and two with a concomitant urinary tract infection. At the onset of gastroenteritis only five patients were fully breast-fed.

Clinical features. The clinical symptoms and signs in the patients with nosocomial and community-acquired gastroenteritis are compared in Table 3. Diarrhea was the most common clinical feature in both groups. Vomiting was reported in approximately 60% of the patients. Fever was more frequent in community-acquired gastroenteritis but it was also reported in 31% of the nosocomial gastroenteritis. Upper respiratory symptoms occurred with equal frequency in both groups.

Three of the dehydrated patients from each group had metabolic acidosis. One of the nosocomial cases had hypertonic dehydration (serum sodium > 145 mmol/liter). Five of the dehydrated patients in both groups (2 of 5 and 3 of 5, respectively) had other fecal pathogens, all of them viruses, concomitantly with astrovirus. One patient excreted both rotavirus and adenovirus 10 days before the onset of gastroenteritis (Fig. 2, Patient 18).

When serial stool samples were examined (Fig. 2)

TABLE 1. Confirmed hospital cases of viral gastroenteritis, September, 1987, to November, 1988

Virus	Nosocomial	Community-acquired
Astrovirus	32 (37)*	13 (7)
Rotavirus	33 (38)	134 (69)
Calicivirus	5 (6)	6 (3)
"Small round structured viruses"	3 (3)	4 (2)
Adenoviruses		
Detected† by electron microscopy	4 (5)	8 (4)
Detected by virus isolation	3 (3)	9 (4)
Enteroviruses‡		
Echovirus types 22, 23	6 (7)	3 (2)
Other enteroviruses	1 (1)	18 (9)

* Numbers in parentheses, percent.

† Adenoviruses that were detected by electron microscopy but not by virus culture were assumed to be enteric adenoviruses, types 40 and/or 41, whereas adenoviruses detected by isolation only were typed and shown to be nonenteric.

‡ The distinction between echovirus types 22 and 23 and other enteroviruses is made because the former are more prone to cause nosocomial spread (A. Ehrnst et al., unpublished data). Fisher's exact test performed on numbers of enteroviruses in the table yields $P = 0.001$.

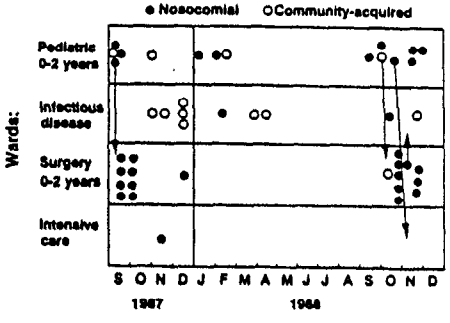

FIG. 1. Cases of astrovirus infections at four wards at St. Göran's Children's Hospital, Stockholm, September, 1987, to December, 1988. *Arrows*, transfer of patients among wards after onset of illness. *Abscissa*, months from September, 1987, to December, 1988.

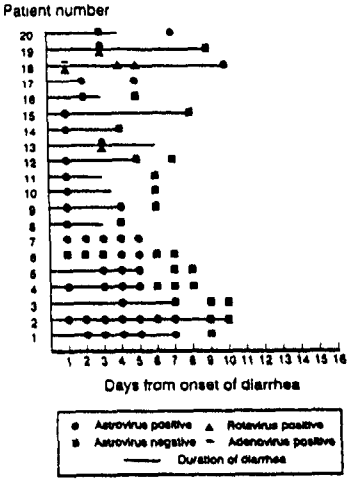

FIG. 2. Duration of diarrhea and stool virus detection in 20 cases of nosocomial astrovirus infection.

TABLE 2. Underlying illnesses in patients with astrovirus infection

	Nosocomial	Community-acquired
Total	32	13
Surgical unit	18	1
Gastrointestinal	11	
Urologic	2	
Trauma	2	1
Cardiologic	1	
Other	2	
Medical unit	10	4
Gastrointestinal	4	
Urinary tract infection		2
Cardiologic	2	
Neurologic	3	
Respiratory	1	
Social		2
Infectious disease unit	2	8
Intensive care	2	
Cardiac	1	
Gastrointestinal	1	

TABLE 3. Symptoms and signs in patients with astrovirus gastroenteritis

	Nosocomial	Community-acquired
Total	32	13
Symptom		
Diarrhea	30 (93)*	12 (92)
Vomiting	21 (66)	7 (54)
Fever ≥38°C	10 (31)	7 (54)
Respiratory symptoms	7 (22)	2 (15)
Dehydration ≥5%	5 (16)	5 (39)
Metabolic acidosis	4 (13)	5 (39)
Duration of symptoms (days)		
Median	4	4
Range	1–10	1–21
Other virus found		
Rotavirus	4	1
Adenovirus	1	2

* Numbers in parentheses, percent

excretion of astrovi ed for 3 to 7 days (median, as calculated from with three or more fecal samples analyzed, 6 days). The duration of clinical illness was 2 to 10 days (median, 4 days). Two children with community-acquired gastroenteritis developed clinical symptoms of transient lactose intolerance of short duration (3 weeks).

Nineteen of the children with nosocomial gastroenteritis either were treated with diet modification or received no treatment. Eleven were given oral rehydration solution either alone or in combination with intravenous fluid. In two patients oral intake was stopped.

DISCUSSION

In the present study the clinical and epidemiologic features of two nosocomial outbreaks of astrovirus infections are described. The age of nosocomially infected cases was significantly younger than that of community-acquired cases. The nosocomial spread of astrovirus infections was more pronounced than that of rotavirus infections. The attack rate of astrovirus infection among cases with gastrointestinal disease was higher than in children with other diseases.

It is interesting that the age of the children with nosocomially acquired astrovirus infection was lower than that of the community-acquired cases. In other studies of nosocomial cases the mean age has also been low.[9, 10, 14, 16] The high incidence in the younger age group among nosocomial cases is most probably a result of the fact that this age group dominates among the hospitalized children present in the wards. Thus there was no age difference between infected and noninfected children in the wards. Astrovirus infections in the community probably occur more often among older children than has previously been fully appreciated. Similarly in a seroepidemiologic survey in the United Kingdom IgM and IgG antibodies to astrovirus were detected not only in infants but also in school children and elderly patients.[17]

The high relative frequency of astrovirus infections is most likely influenced by the time period of the study. It included two outbreaks of astrovirus infection and the interval between them, but only one rotavirus season, between the two astrovirus outbreaks. If both of the astrovirus outbreaks had been omitted from the study, the relative frequency would have been 6.3% (excluding isolation-positive adenovirus infections, and coinfections counted once). In other studies the relative frequency of astrovirus nosocomial diarrhea has been found to be 5.2%[2] and 9.6%.[18] If only 1988 had been included the frequency would have been 35%.

The initial clinical features of astrovirus gastroenteritis were not easily distinguishable from those of rotavirus infection. The median duration of the symptoms was 4 days and the time of excretion was 1 week in accordance with findings by others.[14, 19] In a comparison between outbreaks of rotavirus and astrovirus infections in a geriatric inpatient sample, the duration of rotavirus illness was 4 days and the symptoms were somewhat more severe, but the attack rate of astrovirus infection was higher.[19]

Nosocomial spread seemed to be a feature more of astrovirus than of rotavirus infections. However, this may be biased by the greater number of community-acquired cases of rotavirus infections. This in turn may reflect the mild symptoms of astrovirus gastroenteritis, with most community-acquired cases never seeking medical attention. However, in a study in Glasgow 80% of 62 astrovirus-infected patients had diarrhea whereas 12% were asymptomatic, which was similar to the situation with rotavirus infections.[3] Of verified cases of astrovirus gastroenteritis approximately one-half were found to be nosocomial.[2, 14] Perhaps astroviruses, as has been described for rotavirus, may be transmitted by the air-borne route in addition to being spread by contaminated hands.[20]

No particular risk group of children has been defined in earlier studies, but occasional cases in children with preexisting inflammatory bowel disease have been described.[14] In the present study one-half of the cases with nosocomially acquired astrovirus infection had a gastrointestinal underlying disease. This suggests that gastrointestinal disease is a risk factor.

Both breast milk and orally administered antibodies have been shown to protect against infection and to modify the severity of the illness caused by other viruses.[21–23] In the present study only 6% of those with nosocomial astrovirus were breast-fed. In addition gastrointestinal disease might interfere with the local defense system. These factors may together contribute to a susceptibility to intestinal infection.

Coinfection with astrovirus and other pathogens has been reported to be common. Nazer et al.[14] reported that of 28 children affected by astrovirus 16 excreted other pathogens. In our study 10 patients, either with community-acquired or nosocomial infection, concomitantly excreted other viruses. The children who excreted astrovirus in combination with other viruses were more often dehydrated but the mean duration of illness did not differ from those who excreted astrovirus alone.

Although diarrhea caused by astrovirus may be relatively mild and therefore not strenuous to a nonhospitalized patient, the pronounced tendency to cause nosocomial spread of diarrhea as evidenced by this study and by others[2, 14] is of noteworthy concern to pediatricians.

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Book Review

Infection Control in the Child Care Center and Preschool. Edited by Leigh G. Donowitz. 364 pp. Baltimore: Williams & Wilkins, 1991. Soft cover, $26.00.

This is a great little book. It belongs in every pediatricians's office and in every day-care center. It begins with general information in sections titled Transmission, Policies, Care of High-Risk Children and Guidelines. The real meat of this book is the 54 chapters on specific infections from *Adenovirus* to *Yersinia*. Each chapter is broken into divisions titled Clinical Manifestations, Etiologic Agent, Epidemiology, Diagnosis, Therapy, Infectious Period and Infection Control. Dr. Donowitz has done an excellent job of editing the material into language that any intelligent lay person can understand. Day-care center directors will be able to find out what to do in specific situations and why the measures are necessary. Pediatricians can turn to the book for sound advice from experts when they are asked to advise the day-care center director and parents. This is the most eminently practical and useful book to come along in years. Run to your book store and buy it.

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