Viral and Bacterial Contamination of Multiple-dose Drug Vials Kept in Anesthesia Machines

W. Clayton Petty, Maj., MC,* John P. Heggers, Maj., MSC,†
Dorthy F. Shelton, B.A.,‡ Max K. Mendenhall, Colonel, MC §

U.S.P. regulations require that multiple-dose vials contain antibacterial agents.[1] Anesthesiologists use various multiple-dose vials which, once opened, usually are stored in the anesthesia machine. Some drugs may be used infrequently and remain in the machine drawer for considerable periods. Contamination may occur early in the use of a vial and multiplication of microorganisms can be substantial.

Studies have shown that the neoprene and natural rubber stoppers used in multiple-dose vials can absorb significant amounts of preservative. If the use-life of the multiple-dose vial after manufacture is extended, the preservative may be depleted to a level such that microbiological assay of the vial's contents will show a change.[2, 3, 4] The present study was undertaken to determine if drugs in multiple-dose vials kept in anesthesia machines were contaminated with viral or bacterial agents.

Methods

Two surgical suites in separate buildings were utilized for this study. The drug samples were obtained from partially empty multiple-dose vials in the anesthesia machine drawers. Drugs studied were: (1) atropine sulfate, (2) lidocaine hydrochloride, (3) phenylephrine hydrochloride, (4) succinylcholine chloride, and (5) tubocurarine chloride. Five unopened vials were controls for each drug evaluated. Table 2 lists the five drugs studied, concentrations, vial sizes, preservatives, estimated time of the vial in the anesthesia machine, and the number of samples used in bacterial and viral studies. Samples were taken from the vials using routine aseptic techniques in which the top of the vial is wiped with a sponge saturated with 70 per cent isopropyl alcohol. Separate 2-ml samples for viral and bacteriologic study were withdrawn into a sterile disposable plastic syringe. For bacteriologic study 0.1–0.5 ml was immediately inoculated onto thioglycolate media for incubation. The other sample was frozen and taken directly to the viral laboratory for cell culture inoculation.

Table 1. Viral Agents Studied

1. Picornaviruses
poliomyelitis (types 1, 2, 3)
Coxsackie (A-9, A-16, A-21, A-23, all B's)
ECHO viruses
encephalomyocarditis
rhinoviruses
2. Reoviruses
Three serological types
3. Myxoviruses
influenza A and B
mumps
parainfluenza
4. Adenoviruses
28 serotypes
5. Herpesviruses
herpes simplex

The protocol for the bacteriologic study is shown in outline form in figure 1. All cultures were incubated at 37 C and checked daily for two weeks for growth. Bacteriostatic and bacteriocidal determinations were done utilizing standard dilution methods.

Three types of cells were employed for viral studies: primary human embryonic kidney cells (HEK); stable monkey kidney cells (BSC) and primary baboon kidney cells (BKC). The

* Resident, Department of Anesthesia, Brooke General Hospital.
† Chief, Microbiology Service, Brooke General Hospital.
‡ Supervisory Medical Technologist, Virology Branch, Fourth U. S. Army Medical Lab.
§ Chief, Department of Anesthesia, Brooke General Hospital.
Received from the Department of Anesthesiology, Brooke General Hospital, Fort Sam Houston, Texas 78234.

TABLE 2. Drugs Studied

Drug	Concentration	Vial Size	Preservative	Estimated Time in Anesthesia Machine	No. Samples	
					Bacterial	Viral
Atropine sulfate	0.4 mg/ml	20 ml	Chlorobutanol 0.5 per cent	3 months	30	24
Lidocaine hydrochloride	0.5 per cent	50 ml	Methylparaben 0.01 per cent	14 days	29	25
Phenylephrine hydrochloride	10 mg/ml	5 ml	Phenol USP 0.35 per cent	6 months	23	19
Succinylcholine chloride	20 mg/ml	10 ml	Methylparaben 0.1 per cent Sodium bisulfite 0.1 per cent;	3 days	31	32
Tubocurarine chloride	3 mg/ml	10 ml	Chlorobutanol 0.5 per cent	7 days	28	21
					TOTAL 141	121

majority of viral agents pathogenic to man can be recovered utilizing these three types of cells, as indicated in table 1. Two tubes of each of the three cell cultures were employed for each drug sample. The viral isolation protocol is outlined in figure 2. L-15 medium was used with HEK and BSC cells, with 0.2 ml inoculum and 2.0 ml feedings. Melnick's monkey medium B was used for BKC cells with 0.1 ml inoculum and 1.0 ml feedings. Hemadsorption techniques were used on cells surviving the 14-day observation period. Duplicate *p*H determinations were done on each of the five drugs studied using a Beckman Zeromatic pH meter.

RESULTS

All thioglycolate media for the drugs evaluated were negative for bacterial growth after the two-week observation period. The tenfold serial dilution of each drug inoculated with 0.1 ml of 24-hour broth culture of *Staphylococcus aureus* showed the presence of growth. At 1:2 serial dilution, bacteriostatic and bactericidal studies indicated that succinylcholine and phenylephrine were bactericidal after 24 hours. Tubocurarine and atropine 24-hour colony counts were both 100–1,000, but after five days only 7 and 1, respectively, indicating a bactericidal effect. Table 3 summarizes the bacteriologic data.

All 121 samples submitted for virologic survey failed to show viral contamination. Table 3 lists the results of cell cultures for the five drugs studied.

DISCUSSION

Bacterial contamination was noted in 2.7 per cent of 490 multiple-dose vials studied by Kohan *et al.* in 1962.[5] These vials represented ten different drugs taken from various hospital wards. Samples (27) of atropine showed no contamination. One of four samples of 1 per cent lidocaine (methylparaben 0.01 per cent) showed contamination. Lidocaine (0.5 per cent with methylparaben 0.01 per cent) in our study failed to show any bacterial contamination in 29 samples. Phenylephrine, tubocurarine, and succinylcholine were not studied. The contaminants were identified as common air-borne and skin organisms, leading these authors to conclude that contamination was caused by a break in the chain of sterile technique.

The use of a preservative in a multiple-dose vial is to lend microbiologic stability to the drug. Since the preservative may be absorbed onto the rubber stopper or, as in the case of chlorobutanol, be degraded in solution, the shelf-time of the vial become important.[2, 3, 6] Rapid turnover of vials prevents loss and degradation of preservatives and helps insure resistance to contamination.

Preservatives used to provide bacteriologic stability (no stabilizer known for virus) differ in effectiveness. Succinylcholine, 20 mg/ml, containing 0.18 per cent methylparaben and 0.02 per cent prophylparaben, was studied and the paraben preservatives were found to be satisfactory.[6] The same preservatives were found to be unsatisfactory in the same study

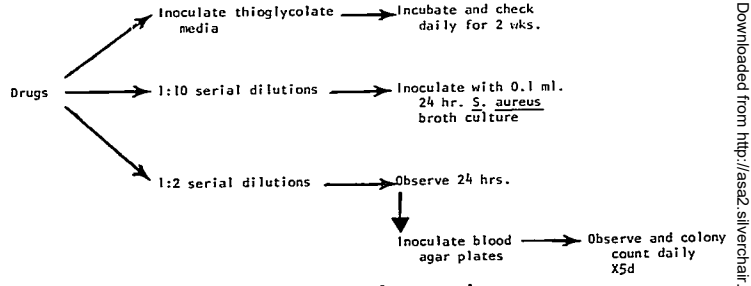

FIG. 1. Bacteriology protocol.

when utilized in multiple-dose vials of estrone aqueous suspension. Use of the correct preservative for each drug is essential for antibacterial action.

LeMar and White[7] found that low *p*H enhances germicidal or bacteriostatic action in ointments. Other studies have shown that lowering of *p*H enhances antimicrobial activity of preservatives, and a *p*H value lower than 2 in itself is a sufficient preservative.[8] Succinylcholine and tubocurarine both are adjusted to *p*H <3 to insure stability of the drug. This low *p*H, therefore, may tend to make them very resistant to bacterial contamination. It is interesting to note that succinylcholine (methylparaben 0.1 per cent) is bactericidal while tubocurarine (sodium bisulfite 0.1 per cent and chlorobutanol 0.5 per cent) is bacteriostatic.

Our review of the literature failed to reveal any recent studies of viral contamination in multiple-dose vials. The threat of viral contamination is of major concern: (1) no preservative is known to be effective against virus, (2) no broad-spectrum antiviral agents for use in man are known, (3) possibilities for viral contamination of drugs are ever present.

CONCLUSIONS

The common practice of using multiple-dose vials kept in anesthesia machines seems safe, especially if they have a rapid turnover rate.

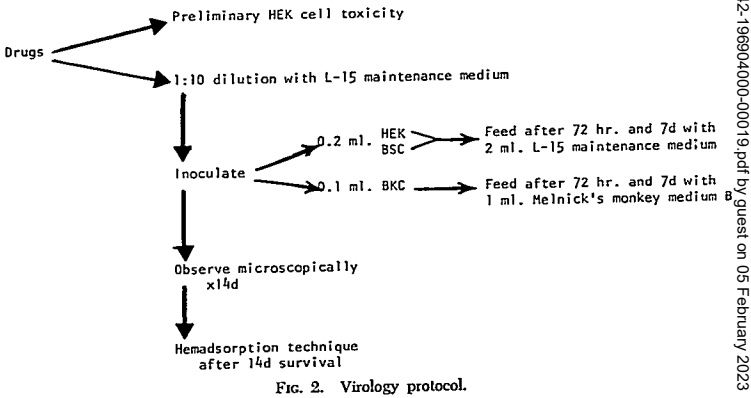

FIG. 2. Virology protocol.

TABLE 3. Viral and Bacterial Studies

Drug	pH	Viral			Bacteriological	
		Cells Employed			Thioglycolate Media	Antibacterial Activity
		HEK	BKC	BSC		
Atropine sulfate	3.69	Neg	Neg	Neg	Neg	Bacteriostatic
Lidocaine hydrochloride	6.11	Neg	Neg	Neg	Neg	None
Phenylephrine hydrochloride	6.13	Neg	Neg	Neg	Neg	Bactericidal
Succinylcholine chloride	2.32	Neg	Neg	Neg	Neg	Bactericidal
Tubocurarine chloride	2.03	Neg	Neg	Neg	Neg	Bacteriostatic

and low *p*H values and are handled with good aseptic technique.

Vials of succinylcholine and phenylephrine are apparently bactericidal, while tubocurarine and atropine are bacteriostatic.

The authors wish to thank Lt. Richard L. Bridwell, C.R.N.A., for his technical assistance.

REFERENCES

1. Martin, E. W. (ed.): Remington's Pharmaceutical Sciences. 13th edition. Easton, Mack Publishing Company, 1965, p. 504.
2. Lachman, L., Weinstein, S., Hopkins, C., Slack, S., Elsman, P., and Cooper, J.: Stability of antibacterial preservatives in parental solution: I. Factors influencing the loss of antimicrobial agents from solutions in rubber-stoppered containers, J. Pharm. Sci. 51: 224, 1962.
3. Lachman, L., Weinstein, S., Urbanyi, T., Ebersold, E., and Cooper, J.: Stability of antibacterial preservatives in parental solution: III. Relationship between chemical loss and microbiological activity in multiple-dose vials, J. Pharm. Sci. 52: 241, 1963.
4. Lachman, L., Urbanyl, T., and Weinstein, S.: Stability of antibacterial preservatives in parental solutions: IV. Contribution of rubber closure composition in preservative loss, J. Pharm. Sci. 52: 244, 1963.
5. Kohan, S., Carlin, H., and Whitehead, R.: A study of contamination of multiple-dose medication vials, Hospitals 36: 78, 1962.
6. Rdzok, E. J., Grundy, W. E., Kirchmeyer, F. J., and Sylvester, J. C.: Determining the efficacy of preservatives in pharmaceutical products, J. Amer. Pharm. Ass. 44: 613, 1955.
7. LeMar, L. E., and White, A. I.: A study of the effect of hydrogen-ion concentration in certain ointments and lotions, J. Amer. Pharm. Ass. 33: 134, 1944.
8. Entrekin, D. N.: Relation of *p*H to preservative effectiveness. I. Acid media, J. Pharm. Sci. 50: 743, 1961.

Simplified Versions of the Shunt and Oxygen Consumption Equations

J. M. LEIGH, M.B., F.F.A.R.C.S.,* M. F. TYRRELL, M.B., F.F.A.R.C.S.,*
D. A. P. STRICKLAND, B.Sc.

When blood traverses the pulmonary circuit some bypasses the gas exchange surfaces. This pulmonary physiologic shunt, or venous admixture effect, is due not only to certain inevitable anatomic pathways but also to a variable amount of ventilation–perfusion inequality. Because of the shunt a negative gradient in oxygen content exists between end-pulmonary-capillary blood and systemic arterial blood. Figure 1 shows the relationships in a conventionally stylized form.

* Recipients of a grant from the Medical Research Council.
Received from the University Department of Anaesthetics and Department of Clinical Measurement, Westminster Medical School, London, England.