(17) J. W. Patton, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1961.
(18) R. I. Ellin, J. S. Carlese, and A. A. Kondritzer, *J. Pharm. Sci.*, **51**, 141(1962).
(19) J. J. O'Neill, H. Kohl, and J. Epstein, *Biochem. Pharmacol.*, **8**, 399(1961).
(20) I. B. Wilson, in "The Enzymes," vol. 4, P. D. Boyer, H. Lardy, and K. Myrbach, Eds., Academic, New York, N. Y., 1961, p. 301.
(21) L. A. Mounter and V. P. Whittaker, *Biochem. J.*, **47**, 525 (1950).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 16, 1971, from the **Biochemistry Section, Basic Medical Sciences Branch, Biomedical Laboratory, Edgewood Arsenal, MD 21010*, and the *†Materials Engineering Lab Section, Physical Chemistry Branch, Chemical Laboratory, Edgewood Arsenal, MD 21010*

Accepted for publication December 10, 1971.

The authors thank Jay Amshey for helpful discussion and editorial comment.

▲ To whom inquiries should be directed.

Survey of Microbial Contamination of Ophthalmic Ointments▲

FRANCES W. BOWMAN, EVERETT W. KNOLL, MACK WHITE, and PHILIP MISLIVEC

Abstract □ A survey was conducted by the Food and Drug Administration to obtain a profile of the microbial load in all antimicrobial ophthalmic ointments manufactured in the United States. Eighty-two batches of ointments from 27 manufacturers were tested. Contamination was detected in 16 batches (19.5%).

Keyphrases □ Ophthalmic ointments—survey of microbial contamination in antibiotic and nonantibiotic commercial preparations □ Microbial contamination of ophthalmic ointments—survey of commercial preparations □ Antibiotic ophthalmic ointments—survey of microbial contamination in commercial preparations □ Sterility of ophthalmic ointments—survey of commercial preparations

The *Antibiotic Regulations* of the Food and Drug Administration (FDA), the USP, and the NF require all ophthalmic solutions and suspensions to be sterile, and they specify sterility tests for these preparations. None of these, however, requires sterility tests for ophthalmic ointments, except for certain antibiotic ophthalmic ointments when these are labeled sterile (1).

From 1947 to 1950, the *Antibiotic Regulations* (2) required a microorganism count to be performed on each batch of antibiotic ophthalmic ointments. (The viable counting method involved smearing the ointment directly on the surface of an agar plate.) During that time, even though the *Regulations* specified that the count should not exceed five viable organisms per gram of ointment, the incidence of contamination detected was very low. This was undoubtedly due to the poor sensitivity of the method, and the test was deleted from the *Regulations* in 1950 (3).

In 1958, Vander Wyk and Granston (4) reported that of 28 samples of antibiotic ophthalmic ointments tested, 17 were nonsterile. The method used included a dispersion step in which the ointment was shaken for 1 hr. at room temperature with sterile glass beads in 25 ml. of distilled water. Three 1.0-ml. aliquots were then placed in petri dishes, mixed with melted blood agar, and incubated at 37° for 24 hr.

The finding of contamination in over 50% of the batches tested prompted a survey by Bowman and Holdowsky (5), who used the same method described by Vander Wyk and Granston (4).

Forty-six samples representing 19 batches of ophthalmic ointments were tested in the survey, and only two batches (10%) were found to be contaminated. Although this method is an improvement over the earlier one (1), its weaknesses are twofold when used to test antibiotic ophthalmic ointments. First, some organisms may become encased in oil or fat and thus be denied access to nutrients required for survival and multiplication. Second, any organisms that may be released from the antibiotic ointment onto the culture medium encounter the antimicrobial activity of the antibiotic from the product, and this activity might prevent their growth. Thus, a better method for use with petrolatum-based ointments is needed.

The use of isopropyl myristate as a diluent for petrolatum-based ointments was first reported by Sokolski and Chidester (6), who improved the recovery of viable cells from ointments by a filtration technique. Isopropyl myristate dissolves certain sterile petrolatum-based antibiotic ointments so that these preparations can be tested for sterility by membrane filtration. With the advent of this improved procedure for recovering microorganisms from petrolatum-based ointments, another survey was conducted in 1968 to reinvestigate the incidence of microbial contamination in antibiotic ointments. Although contamination was detected in eight batches (7%) of the 114 batches tested, no *Pseudomonas aeruginosa* or any other Gram-negative bacilli were recovered.

In 1964, eight cases of severe eye disorder were reported in Sweden in workmen treated with a contaminated antibiotic ointment (7). As a result of the infections, one patient lost an eye and the other patients suffered reduced visual acuity. *P. aeruginosa* of identical characteristics was isolated both from the infected eyes and from the ointment used in the treatment. Since this unfortunate incident, Sweden has imposed a sterility requirement on ophthalmic ointments. Similar un-

fortunate happenings involving nonsterility of eye preparations in Great Britain led to the introduction of a sterility test for eye ointments in the 1968 "British Pharmacopoeia" (8).

These findings prompted FDA to conduct a survey on all ophthalmic ointments (antibiotic and nonantibiotic) manufactured in the United States to obtain a profile of the microbial load in such ointments presently on the market.

EXPERIMENTAL

Sample Collection—FDA district offices throughout the United States collected samples from all known manufacturers of ophthalmic ointments. In addition to collecting the samples, the inspectors inspected the manufacturing and control facilities of each firm. Sixty units of the finished product of each batch of ointment sampled were sent to these laboratories for microbial evaluation. This number allowed for a retest on 40 units if the first 20 units tested were found to be contaminated. Eighty-two batches of ointments from 27 manufacturers were received.

Mercurial-Containing Ointments—Nine batches contained mercurial compounds as the active ingredients. Since the authors had no prior experience in testing for the sterility of petrolatum-based ointments containing mercurials, some investigational studies were performed. The membrane filtration procedure used was unable to recover bacterial or mold spores from these ointments because of the inhibitory effect of the high concentration of mercury compounds remaining on the membrane. Low concentrations of mercurial preparations can be inactivated with 0.05% w/v sodium thioglycolate. However, it was impossible to neutralize the antimicrobial effects of the mercury compounds left on the membranes by washing with sodium thioglycolate solutions or by using a thioglycolate culture medium. Since the mercurials in these ointments were sporistatic rather than sporicidal, the results thus obtained could very well be false negatives.

Plastic-Based Ointments—In addition to batches containing mercurials, the test as applied to five other batches was somewhat unsatisfactory because they were plastic-based ointments rather than petrolatum-based ointments. Because plastic-based ointments do not dissolve in isopropyl myristate, they must be tested by the less sensitive direct method. Only microorganisms that are highly resistant to the antimicrobial agents or preservatives contained in the formulation can be detected.

Petrolatum-Based Ointments—For soluble antibiotic ointments, a method based on the one described in Section 141e.433 of the *Antibiotic Regulations* (1) was used. For nonantibiotic ointments soluble in isopropyl myristate, however, the method described in USP XVIII (9) was used[1].

Membrane Filtration—A three-place filtration manifold was used for filtrations. The manifold, which supports three 250-ml. plastic filter funnels, combines a vacuum manifold and a flushing manifold to make a complete sterility test system. Obviously, these tests could also have been performed with individually mounted filter holders. In the beginning, plastic filter funnels were used exclusively for the tests because after an ointment filtration the plastic bases are easier to clean than the fritted glass bases of the Pyrex funnels. However, their use for testing was abandoned when it was discovered that the plastic failed to withstand repeated autoclaving after being in contact with isopropyl myristate. The combination of isopropyl myristate and the high temperature of the sterilizer caused the threads of the funnel to crack open and the plastic to become opaque. Therefore, the Pyrex filter holders were substituted for the plastic filter units.

Membranes—Since the filtration of isopropyl myristate solutions of oils or products containing oil requires a water-free system, it was necessary to sterilize the membranes separately rather than mounted in the manifolds. Membranes wrapped in packages of 100 were autoclaved at 121° for 10 min., and the manifolds with the Pyrex funnels were autoclaved at 121° for 30 min.

Isopropyl Myristate—Recently, there has been some concern over the possibility that isopropyl myristate might be toxic for viable microorganisms. Some discrepancies found in the recovery rates between various laboratories may be due to the source or grade of isopropyl myristate. In 1969, Dony (10) reported that an International Study Group on Microbial Purity of Drugs discontinued using isopropyl myristate because of its rapid bactericidal action. At the authors' suggestion, the Study Group used isopropyl myristate from a different source and found that it was satisfactory for recovering microorganisms from oily preparations.

In their investigational studies, Tsuji *et al.* (11) obtained isopropyl myristate from several different suppliers[2]. It was sterilized either by dry heat (100-ml. portions were heated in 250-ml. conical flasks at 80° for 4 hr.) or by filtration (the samples were filtered through a 0.22-μ bacteria-retentive membrane filter). These studies revealed no difficulties that could be attributed to the grade or source of the isopropyl myristate. However, the method of sterilization revealed significant differences in the antimicrobial activity of the isopropyl myristate. Tsuji *et al.* (11) calculated the *D* values (time in minutes required to kill 90% of the microorganisms) for five microorganisms and found that the filtered isopropyl myristate is significantly less toxic than the heat-sterilized isopropyl myristate.

In an effort to determine the cause of the increased toxicity of the heat-sterilized isopropyl myristate, Tsuji *et al.* (11) compared its acidity to that of the filter sterilized. The pH's were measured on extracts prepared by adding water to the isopropyl myristate and shaking for 1 hr. The pH of the extract from the heat-sterilized isopropyl myristate was 3.3, whereas that from the filter sterilized was 5.0. They postulated that the increase in toxicity of the heat-sterilized isopropyl myristate may be due to the increase in acidity. Their findings were confirmed by this laboratory; therefore, all the isopropyl myristate used in this survey was filter sterilized.

RESULTS

Sterility Tests—Eighty-two batches of ophthalmic ointments from 27 manufacturers were evaluated for microbial contamination. Sixteen batches (19.5%) were found to be contaminated on both the first and second tests. Of the 35 batches of antibiotic ointments tested, 23% were found contaminated; of the 47 batches of nonantibiotic ointments, 17% were found contaminated. No contamination was found in the nine batches of ointments containing either mercuric oxide or bichloride of mercury. As noted previously, negative results in these tests do not indicate conclusively that they are sterile. As shown in Table I, 16 batches contained molds. In addition to the mold contamination, three batches also contained yeast and three other batches contained yeast and *Bacillus* sp. The level of contamination found was low, *i.e.*, 1–10 microorganisms/g. ointment.

Characteristics of Molds Recovered—The molds were identified according to their morphology and growth characteristics on Czapek-Dox, malt, and potato dextrose agar media. *Aspergillus flavus* was recovered from four batches of ointment from different manufacturers. Each isolate was tested for its ability to produce aflatoxins as described by Mislivec *et al.* (12), since some strains of *A. flavus* produce these potent, naturally occurring carcinogens. Chloroform extracts of one of the isolates showed substantial amounts of aflatoxin B_1 and B_2 when tested by TLC. The other three isolates of *A. flavus* did not produce aflatoxins.

Penicillium chrysogenum, some strains of which produce penicillin, was recovered from five batches of ointments. In recent investigations by Banks *et al.* (13), seven penicillin-producing strains of *P. chrysogenum* were found to contain viruslike particles. One isolate of *P. chrysogenum* was screened for the presence of virus, but no virus was found.

Whether the molds recovered from the ointments can be considered as inherently pathogenic or merely as opportunistic pathogens is a moot question. Vaughan and Riegelman (14) claimed that although corneal ulcers are not common, there has been an undeniable increase of incidence since the advent of corticosteroids and antibiotics. To support their position, they cited the isolation of 13 fungi from corneal ulcers in the 10-year period of 1953–1963 at the University of California Hospital. The fungi were identified by microscopic examination of stained smears and cultures.

[1] This method was revised in the "First Supplement to USP XVIII," Oct. 1971.

[2] Givauden-Delawanna, Inc., New York, as Deltyl Extra: Goldschmidt Chemical Division of Wilson Pharmaceutical, New York; and M. W. Parsons-Plymouth Division of S. B. Penick & Co., New York.

Table I—Results of Evaluation of Ophthalmic Ointments for Microbial Contamination

Active Ingredients	Quantity	Number of Batches		Organisms Recovered from Contaminated Batches
		Negative	Positive	
Nonantibiotic Ointments				
Boric acid	5%	2	0	—
Boric acid	10%	2	0	—
Yellow mercuric oxide	1%	2	0	—
Boric acid	—	—	—	—
Zinc sulfate	—	—	—	—
Yellow mercuric oxide	1%	4	0	—
Yellow mercuric oxide	2%	3	0	—
Atropine sulfate	1%	1	1	*Aspergillus niger*, *Bacillus* sp.
Atropine sulfate	1%	0	1	Yeast
Chlorobutanol	0.5%	—	—	Sphaeropsidales (order)
Atropine alkaloid	0.5%	0	1	*Aspergillus niger*, *Aspergillus flavus*
Atropine	0.5%	1	0	—
Glycolmonostearate	—	—	—	—
Chlorobutanol	0.25%	2	0	—
Boric acid	5.0%	—	—	—
Sodium chloride	5%	1	0	—
Tetracaine	5 mg.	1	0	—
Vitamin A	1500 units	2	0	—
Vitamin D	200 units	—	—	—
Piperocaine	4%	1	0	—
Idoxuridine	5 mg.	1	0	—
Isoflurophate	0.025%	1	0	—
Prednisolone	0.025%	1	0	—
Sodium phosphate	—	—	—	—
Hydrocortisone acetate	1.5%	0	1	Yeast, *Bacillus* sp., *Aspergillus versicolor*
Epinephrine	1:50000	1	0	—
Phenacaine	1%	—	—	—
Sulfathiazole	5%	1	1	Yeast, *Bacillus* sp., *Aspergillus niger*
Sulfadiazine	5%	0	1	Yeast, *Penicillium olivino-viride*
Sulfisoxazole	4%	1	0	—
Phenylmercuric nitrate	1:50000	—	—	—
Sodium sulfacetamide	100 mg.	1	0	—
Sodium sulfacetamide	100 mg.	—	—	—
Prednisolone acetate	5 mg.	0	1	*Bacillus* sp.
Sulfanilamide	5%	2	0	—
Phenacaine	1%	—	—	—
Sodium sulfacetamide	10%	1	0	—
Methylparaben	0.5%	—	—	—
Propylparaben	0.1%	—	—	—
Dexamethasone sodium phosphate	0.05%	1	1	*Aspergillus niger*, *Aspergillus flavus*
Physostigmine	0.25%	1	0	—
Mercury chloride	1:3000	2	0	—
Sodium chloride	5:3000	—	—	—
Physostigmine salicylate	0.25%	1	0	—
Butacaine sulfate	2%	1	0	—
Nitromersol	0.03%	—	—	—
Sodium sulfacetamide	10%	—	—	—
Prednisolone acetate	0.25%	1	0	—
Methylparaben	0.5%	—	—	—
Propylparaben	0.1%	—	—	—
Total nonantibiotic batches	—	39	8	—
Percent contaminated	—	—	17.0%	—
Antibiotic Ointments				
Potassium penicillin	1000 μ	2	0	—
Potassium penicillin	2000 μ	1	0	—
Tetracycline hydrochloride	1%	1	0	—
Tetracycline hydrochloride	1%	1	0	—
Hydrocortisone	1.5%	—	—	—
Chlortetracycline hydrochloride	1%	1	0	—
Oxytetracycline hydrochloride	5 mg.	1	0	—
Polymyxin B sulfate	10,000 μ	—	—	—
Polymyxin B sulfate	5000 μ	1	0	—
Chloramphenicol	1%	—	—	—
Polymyxin B sulfate	5000 μ	1	0	—
Chloramphenicol	1%	—	—	—
Hydrocortisone	0.5%	—	—	—
Chloramphenicol	1%	1	0	—
Bacitracin	500 μ	1	0	—
Phenacaine	2%	—	—	—
Neomycin sulfate	3.5 mg.[a]	1	0	—

Table I—(*continued*)

Active Ingredients	Quantity	Number of Batches—Negative	Number of Batches—Positive	Organisms Recovered from Contaminated Batches
Dexamethasone	0.05%	—	—	—
Neomycin sulfate	3.5 mg.[a]	1	0	—
Triamcinolone acetonide	0.1%	—	—	—
Neomycin sulfate	2.5 mg.	1	0	—
Gramicidin	0.25 mg.	—	—	—
Fludrocortisone acetate	1.0 mg.	—	—	—
Neomycin sulfate	3.5 mg.	1	0	—
Polymyxin B sulfate	6000 μ	—	—	—
Dexamethasone	0.1%	—	—	—
Methylparaben	0.5%	—	—	—
Propylparaben	0.01%	—	—	—
Bacitracin	500 μ	4	0	—
Bacitracin	500 μ	1	0	—
Polymyxin B sulfate	5000 μ	—	—	—
Neomycin sulfate	3.5 mg.	—	—	—
Bacitracin	500 μ	0	1	*Penicillium chrysogenum*
Polymyxin B sulfate	10,000 μ	—	—	*Alternaria tenuis*
Neomycin sulfate	3.5 mg.	—	—	*Epicoccum* sp.
Bacitracin	500 μ	1	0	—
Polymyxin B sulfate	10,000 μ	—	—	—
Neomycin sulfate	3 mg.	—	—	—
Hydrocortisone	1%	—	—	—
Bacitracin	400 μ	1	0	—
Polymyxin B sulfate	5000 μ	—	—	—
Neomycin sulfate	3.5 mg.	—	—	—
Hydrocortisone acetate	1%	—	—	—
Neomycin sulfate	3.5 mg.	1	2	No. 1—*Aspergillus niger*, *Penicillium chrysogenum* No. 2—*Aspergillus flavus*, *Penicillium citrinum*
Bacitracin	400 μ	1	4	No. 1—*Aspergillus nidulans*, *Penicillium chrysogenum*
Polymyxin B sulfate	5000 μ	—	—	
Neomycin sulfate	3.5 mg.	—	—	No. 2—*Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium oxalicum* No. 3—*Penicillium chrysogenum*, *Penicillium citrinum*, Yeast, *Bacillus* sp. No. 4—*Aspergillus flavus*, *Penicillium citrinum*
Neomycin sulfate	3.5 mg.	0	1	*Aspergillus niger*
Cortisone acetate	15 mg.	—	—	*Epicoccum* sp., Yeast
Neomycin sulfate	3.5 mg.	1	0	—
Hydrocortisone acetate	15 mg.	—	—	—
Neomycin sulfate	3.5 mg.	2	0	—
Hydrocortisone acetate	5 mg.	—	—	—
Neomycin sulfate	3.5 mg.	1	0	—
Hydrocortisone	5 mg.	—	—	—
Total antibiotic batches	—	28	8	—
Percent contaminated	—	—	22%	—

[a] All neomycin sulfate potencies are equivalent to neomycin base per gram.

CONCLUSIONS

1. For the sterility testing of oleaginous preparations, glass filter funnels and bases are recommended.
2. The need exists for chemical specifications for isopropyl myristate that could be used as criteria for acceptance of the solvent for use in sterility testing.
3. The results of this survey show that many ophthalmic ointments are undesirably contaminated.
4. The contamination recovered from antibiotic ointments confirms the results of earlier surveys (5).

REFERENCES

(1) "Code of Federal Regulations," Title 21, Parts 130 to end (revised Jan. 1, 1970), Section 141e.433, p. 239.

(2) *Fed. Regist.*, **12,** 2223(1947).

(3) *Ibid.*, **15,** 4976(1950).

(4) R. W. Vander Wyk and A. E. Granston, *J. Amer. Pharm. Ass., Sci. Ed.*, **47,** 193(1958).

(5) F. W. Bowman and S. Holdowsky, *ibid.*, **48,** 95(1959).

(6) W. T. Sokolski and C. G. Chidester, *J. Pharm. Sci.*, **53,** 103 (1964).

(7) L. O. Kallings and L. S. Ringerty, *Acta Pharm. Suecica*, **3,** 219(1966).

(8) "The British Pharmacopoeia," General Medical Council, The Pharmaceutical Press, London, England, 1968, p. 409.

(9) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970.

(10) I. Dony, Federation International Pharmaceutique, Brussels, Belgium, personal communication, Nov. 1969.

(11) K. Tsuji, E. M. Stapert, J. H. Robertson, and P. M. Waiyaki, *Appl. Microbiol.*, **20,** 798(1970).

(12) P. B. Mislivec, J. Hunter, and J. Tuite, *ibid.*, **16,** 1053(1968).

(13) G. T. Banks, K. W. Buck, E. B. Chain, J. E. Darbyshire, and F. Himmelweit, *Nature*, **222,** 89(1969).

(14) D. G. Vaughan and S. Riegelman, in "Ocular Pharmacology and Therapeutics and the Problems of Medical Management," S. J. Kimura and E. K. Goodner, Eds., F. A. Davis, Philadelphia, Pa., 1963.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 30, 1971, from the *National Center for Antibiotics Analysis, Office of Pharmaceutical Research and Testing, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC 20204*

Accepted for publication December 14, 1971.

▲ Inquiries should be directed to: Helen L. Reynolds, Bureau of Foods, Food and Drug Administration, Washington, DC 20204