ture. All the compounds were isolated from the reaction mixture as the dihydrochlorides; they are colorless crystalline solids, and their properties are reported in Table II.

Preliminary data on the pharmacological screening which was performed in accordance with the techniques previously described (11), have shown that some members of both series possess a certain degree of antispasmodic, local anesthetic, and antitussive activity.

EXPERIMENTAL

Boiling points are uncorrected. Melting points are corrected and were taken on a Büchi capillary melting point apparatus. The intermediates were commercial products or else obtained according to the procedures reported in the literature.

Typical preparations of both the esters and the acids are illustrated in the following examples.

N - (3 - Dimethylaminopropyl)-alanine Ethyl Ester (XIV).—Ethyl α-bromo-propionate (54.3 Gm., 0.3 mole) dissolved in ether (60 ml.) was added dropwise to a solution of 3-dimethylamino-1-propylamine (30.6 Gm., 0.3 mole) and triethylamine (30.3 Gm., 0.3 mole) and ether (150 ml.), stirring and cooling moderately to room temperature. The mixture was stirred for 1 hr., then refluxed for 2 hr., and allowed to stand overnight. The precipitated triethylamine hydrobromide was filtered off and the solvent removed under reduced pressure. The residue was then distilled, b.p. 72–74° (1 mm.), giving a colorless oil (41.9 Gm.).

N - (2 - Pyrrolidinylethyl) - glycine Dihydrochloride (XVIII).—A mixture of IV (20 Gm., 0.1 mole), sodium hydroxide (6 Gm., 0.15 mole), and water (24 ml.) was cautiously heated to 65°, with efficient stirring. At this temperature hydrolysis continued spontaneously, without necessitating further heating. The solution so obtained was washed with ether and acidified to pH 1 by cautious addition of concentrated hydrochloric acid. The reaction mixture was evaporated to dryness *in vacuo* and the residue was extracted with 300 ml. of boiling ethanol in portions. The combined alcoholic extracts were then distilled and the residue (19.8 Gm.) was crystallized from 98% ethanol. After drying at 90° *in vacuo*, colorless crystals were obtained, m.p. 190–191° dec.

REFERENCES

(1) Goldin, A., Dennis, D., Venditti, J. M., and Humphreys, S. R., *Science*, **121**, 364(1955).
(2) Edwards, B. K., Goldberg, A. A., and Wragg, A. H. *J. Pharm. Pharmacol.*, **12**, 179(1960).
(3) Shapero, M., and Edwards, K. B., *ibid.*, **14**, 119(1962).
(4) Rips, R., Derappe, C., and Magnin, P., *J. Med. Chem.*, **8**, 529(1965).
(5) Tien, N. B., Buu-Hoi, Ng. Ph., and Xuong, Ng. D., *J. Org. Chem.*, **23**, 186(1958).
(6) Finger, G. C., Dickerson, D. R., Starr, L. D., and Orlopp, D. E., *J. Med. Chem.*, **8**, 405(1965).
(7) Bruzzese, T., Casadio, S., Marazzi-Uberti, E., and Turba, C., *J. Pharm. Sci.*, **54**, 1041(1965).
(8) Speziale, A. J., and Jaworski, E. G., *J. Org. Chem.*, **25**, 728(1960).
(9) Fugger, J., Tien, J. M., and Hunsberger, J. M., *J. Am. Chem. Soc.*, **77**, 1843(1955).
(10) Greco, C. V., Nyberg, W. H., and Cheng, C. C., *J. Med. Pharm. Chem.*, **5**, 861(1962).
(11) Casadio, S., Bruzzese, T., Pala, G., Coppi, G., and Turba, C., *J. Med. Chem.*, to be published.

Effect of Germicidal Aerosol Treatment on the Microbial Flora of Laboratory Air

By LEO GREENBERG

Metered doses of a quaternary ammonium, glycol, alcohol formulation were dispensed automatically by motorized apparatus at 15-min. intervals around the clock for 1 month, and the effects of such treatment on the microbial flora of the air in a heavily trafficked area were studied. Data indicate that, despite large variations in number and types of microorganisms found in the air, especially during periods of heavy traffic, continued aerosol treatment was capable of sharply reducing mean population values and in altering the flora from a predominantly bacterial population to one dominated by members of the *Penicillium–Aspergillus* group of fungi.

In recent years, the subjects of microbiological air pollution and air sanitation have gained considerable importance, and much information is now available concerning the immediate and latent effects caused by inhalation and retention of foreign airborne particles and bacteria. Present knowledge indicates that particles approximately 1–5 μ in diameter are most effective for penetration and retention in the deep pulmonary spaces (1), and in addition, larger particles bearing many organisms may infect open wounds. In light of recent experiences with hospital-associated staphylococcic infections, much attention has been devoted to the removal or inactivation of biological particles of all sizes from the air used in critical spaces.

To accomplish these ends, a widely diversified group of chemical agents and methods has been proposed, including the use of the gaseous fumigants formaldehyde, β-propiolactone, and ethylene oxide. For more limited and routine use, numerous commercial products designed to reduce air contamination have been developed, and are designed for aerosolization either by mechanical spray or by means of propellants such as the freons. Among the agents utilized for such purposes have been various alcohols, glycols, volatile oils, phenols, and quaternaries.

In general, such commercial aerosols have been

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Table I.—Colony Counts of Microorganisms Collected by Air Sampling in Test Laboratory

Organism	(Start) No Treatment, 2 Wk.[a]	Aerosol Treatment Wk. 1	Wk. 2	Wk. 3	Wk. 4	(Finish) No Treatment 2 Wk.[a]
Bacteria	2176	1559	820	463	148	413
Cocci	587	280	124	38	7	37
Gram-positive spore-formers	1306	1060	588	342	124	347
Other	283	219	108	83	17	29
Yeasts	80	84	49	1	9	13
Molds	398	463	771	666	669	906
Penicillium–Aspergillus	62	89	266	271	308	413
Alternaria	17	29	35	34	38	50
Others	219	345	470	361	323	443
Total colonies	2654	2106	1640	1130	826	1332

[a] Mean weekly value.

evaluated either by determining minimum inhibitory concentrations of active ingredients against standard suspensions of known microbial species or through the use of closed chamber techniques in which a synthetic atmosphere is produced which contains a known concentration of microbial forms, either vegetative or spores. *Serratia indica* and *Bacillus subtilis* var. niger (*Bacillus globigii*) are among the organisms more commonly employed for study (2). In either case, results obtained have little in common with actual conditions of use in a critical air space such as a hospital room or laboratory. The present study was undertaken to determine the effects of germicidal aerosol treatment on the microbial flora of the air in a heavily trafficked laboratory over an extended period of time.

MATERIALS AND METHODS

The laboratory chosen for study was approximately 30,000 cu. ft. with two windows at one end and a door at the opposite end. The only furniture at the beginning of the experiment consisted of several long, formica-topped tables. Table tops, floor, walls, ledges, and other exposed surfaces were cleaned by normal maintenance prior to the start of the experiment.

The aerosol formulation chosen for study contained 1.8% quaternary ammonium compounds as BTC-50[1] in an alcohol–glycol base, and a fluorinated hydrocarbon[2] propellant. A special metering valve was installed on all containers to provide for dispensing a 100-mg. spray dose at each activation. Quantitative studies indicated that such an arrangement produced a spray with a median particle size of 18 μ and that each 100-mg. spray contained approximately 12,000,000 aerosol particles.

To provide constant round-the-clock air treatment, a special dispenser was adapted to activate the container valve at 15-min. intervals. The unit was powered by a small, synchronous motor similar to that of an electric clock, operating on 115 v. 60 cycle a.c. Preliminary investigation determined that 15-min. intervals represented the most satisfactory spacing of spray bursts. Such spacing yielded barely measurable levels of germicidal particles at the end of the period but did not permit excessive build-up of concentrations in daily use.

[1] Supplied by Onyx Chemical Co.
[2] Marketed as Freon-12 by E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.

Three units were installed on the walls of the laboratory 8 ft. above the floor level so spaced that the spray paths converged in the approximate center of the laboratory. Air samples were collected every 4 hr. during the day and at 6-hr. intervals during the night for 8 weeks. To determine pretreatment normal values, nonmedicated aerosol was employed during the first 2 weeks. Medicated aerosol was sprayed during the next 4 consecutive weeks and finally, during the next 2 weeks the nonmedicated spray was used again.

Air sampling in duplicate was done by means of a hospital contamination analysis kit[3] utilizing impingement to buffered gelatin broth and concentration and cultivation on filter disks. Samples were taken for a 5-min. period at an approximate flow rate of 12.5 L. of air per minute. Brain heart infusion agar was used for total aerobic bacterial counts while potato dextrose agar, acidified with sterile 10% tartaric acid to a pH of 3.5 was used for mold and yeast determinations (3).

Throughout the study, a constant traffic pattern was established; students entered and left at will during the hours of 10 a.m. and 4 p.m. Windows and doors were closed at other times, except for the entrance and exit of the sample taker. Parallel samples were taken in an adjoining laboratory to confirm that any gross changes in flora observed during the 8-week experiment were not simply seasonal variations in the general environment. Room temperature, relative humidity, and barometric pressure were recorded as each air sample was taken to determine if these factors seemed to influence the results.

Bacterial colony counts were made after 48-hr. incubation at 35° while yeast and mold counts were made after 7-day incubation at 32°. Following counting, simple microscopic and macroscopic criteria were used to single out the following groups of organisms: sarcina, staphylococcus types (micrococci), Gram-positive spore-forming bacilli, yeasts, and molds. Among those colonies identified as molds, members of the general *Penicillium*, *Aspergillus*, and *Alternaria* were identified routinely by conidial characteristics and *Mucor*, *Neurospora*, *Cladosporium* and other common forms diagnosed where possible by morphological characteristics.

[3] Millipore Filter Corp., Bedford, Mass.

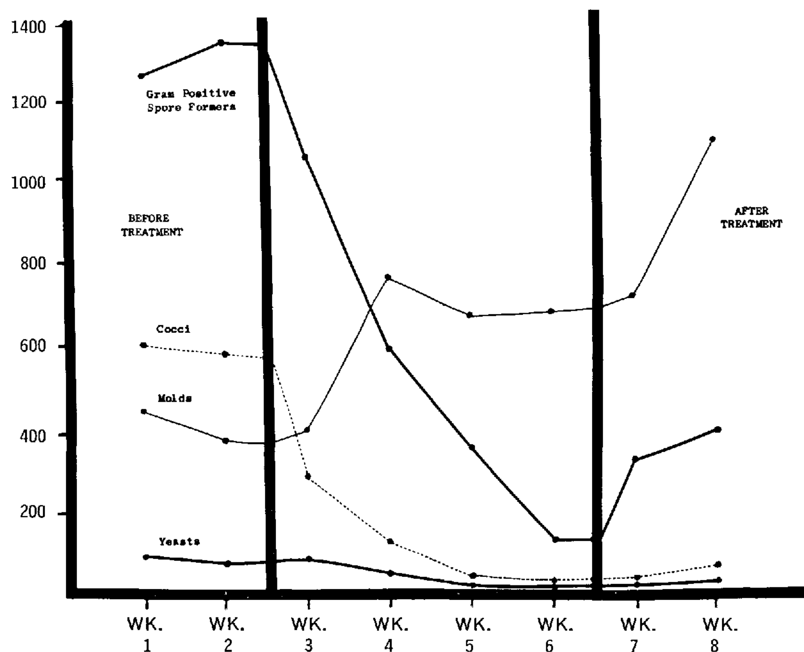

Fig. 1.—Average weekly number of colony isolates identified as Gram-positive spore-forming bacilli, cocci, molds, and yeasts.

RESULTS AND DISCUSSION

Monitoring the air during the preliminary 2-week period in both the test laboratory and the control laboratory indicated that the normal flora in this particular environment consisted of an over-all mean distribution as follows: bacteria, 82% (range 48–96%); yeasts, 3% (range 0–5%); molds, 15% (range 2–28%). As might well have been expected, mean values for total bacterial counts tended to rise sharply during periods of heavy traffic. However, values did not fall promptly on cessation of traffic. For example, during this 2-week period, the number of bacterial colonies isolated rose from 227 at 8 a.m. to 492 at 12 noon and fell only to 451 at 8 p.m. On more than half of the days sampled, total bacterial counts were higher 4 and 8 hr. after traffic periods than during traffic periods. A similar effect, although less marked, was found in total mold counts. This persistence was most evident among the penicillia and almost nonexistent with yeasts.

With the onset of air treatment, profound alterations in the microbial flora were observed which are summarized in Table I and graphically represented in Fig. 1. These changes were not found in values obtained in the control environment and may be regarded as being the result of the spray regimen rather than as normal or seasonal variations. Although there were marked fluctuations in experimental values at different times during the 4-week experimental period, the general trend was clearly toward a marked drop in total microbial species amenable to routine cultivation, and a pronounced conversion of the persistent flora from one dominated by Gram-positive spore-forming bacilli to one dominated by the *Penicillium–Aspergillus* group of molds. Among the bacteria, a relatively rapid decline in the presence of coccal forms apparently testifies to the sensitivity of such species to quaternary germicides, and the sharp quantitative drop in yeast isolates may be similarly explained.

No evident correlation could be discerned between the alterations in flora recorded during the course of the experiment and the temperature, barometric pressure, or relative humidity readings, although there remains with the experimenter the subjective impression that mold colony counts, particularly in the *Penicillium–Aspergillus* group, tended to increase during periods of relatively high humidity.

In conclusion, it seems evident that the use of germicidal aerosol is capable of changing both the quality and quantity of the microbial flora of the air even under conditions of free access and air exchange in the space evaluated. The use of a quaternary type disinfectant system is purely a personal choice, and it is probable that where air sanitation of critical spaces is desired and for one reason or another the quaternary ammonium type of germicide is undesirable, satisfactory results can be obtained with other formulations. The apparent success of the present system against coccal forms may indicate a clinical usefulness against pathogenic forms of staphylococci, currently of profound public health significance, and if such is the case, it is encouraging to note from the data that cessation of air sanitation under the conditions described did not result in the prompt re-establishment of the pretreatment flora.

REFERENCES

(1) Langmuir, A. D., *Am. J. Public Health*, **54**, 1666(1964).
(2) Decker, H. M., Buchanan, L. M., Hall, L. B., and Goddard, B. S., *ibid.*, **53**, 1982(1963).
(3) Kotula, A. W. and Kinner, J. A., *Appl. Microbiol.*, **12** 179(1964).