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Ozone Decontamination of Bioclean Rooms

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To establish a convenient method for decontaminating bioclean rooms, the effect of ozone at 80 mg/m^3 for 72 h was compared with formaldehyde vaporization at an initial concentration of 150 mg/m^3 with a gradual decrease to 20 mg/m^3 during 72 h. Ozone was found to be inferior to formaldehyde in activity. When the bioclean room was decontaminated twice with ozone, the mean colony count per 10 cm^2 was decreased to about the same level as when formaldehyde was used. Ozone had a strong caustic effect upon rubber materials. Despite these disadvantages, ozone decontamination was demonstrated to be superior to formaldehyde vaporization because of convenience, insignificant inhalation of the disinfectant by the hospital staff, and very rapid expulsion of the gas after ventilation. Because the disadvantages of ozone can be easily controlled, this study suggests that ozone decontamination is a promising method for maintaining bioclean rooms.

Bioclean rooms are commonly used for the treatment of patients with acute leukemia and other diseases with a great risk of infection, but the nursing procedures, operation and maintenance of bioclean rooms have not yet been well defined. Studies have been made to help alleviate the expenses and inconvenience of maintaining such rooms.

Decontamination is one of the practical problems involved in maintaining bioclean rooms, and many hospitals use formaldehyde vaporization, peracetic acid, chlorhexidine, or organic tin compound (Biomet 611) spray (1) for this purpose. These procedures, however, have a number of disadvantages, such as inhalation of the disinfectant vapors by the hospital staff, the formation of dirty flecks on glass surfaces, and the retention of unpleasant disinfectant odor after decontamination.

Ozone has been reported to be useful in the decontamination of water (4) and contact lenses (5). One of the major advantages is that the release of ozone can be controlled from outside the room.

This report presents data on the use of ozone for decontaminating bioclean rooms in comparison with data on the use of formaldehyde vaporization.

MATERIALS AND METHODS

Three bioclean rooms (3.4 by 3.4 by 2.1 m) with horizontal laminar airflow were used in this study. After a general cleaning of the rooms before the admission of patients, two rooms were decontaminated with ozone, and one room was decontaminated with formaldehyde gas for 72 h. These are referred to as the ozone 1 or 2 experiment and the formaldehyde experiment, respectively. After the ozone 1 experiment, the room was again decontaminated with ozone, this being referred to as the ozone 1′ experiment.

The bioclean rooms could be sealed so that they were airtight by taping the doors and were equipped with ventilating fans which could be controlled from outside the rooms.

Ozone generators. Three UV tubes (Suv-110; Ikiken Co., Tokyo) were used as ozone generators; they could generate ozone at a concentration of 824 mg/m^3 at the edge of a quartz glass tube 28 mm in diameter at 10 liters/min aeration.

During decontamination, the laminar airflow was turned off. In the preliminary experiment, the ozone concentration could be decreased from 136 to 49 mg/m^3 in 10 min by turning on the laminar airflow.

Formaldehyde vaporization. Potassium permanganate (500 g) was dissolved in 1 liter of water, and 1 liter of 35% Formalin aqueous solution was added.

The ozone concentration was measured continuously by the chemiluminescent method (Japanese Industrial Standard B. 7951, 1976) with a model GLX II ozone meter (Denkikagaku Keiki Co., Ltd., Tokyo). The formaldehyde concentration was measured by the detector tube method with a reactive tube containing xylene adsorbed on silica gel and a detector tube containing sulfuric acid adsorbed on silica gel grains (6). The temperature and relative humidity of the room were recorded continuously with a bimetal and hair automatic recording hygrothermograph (Ota Keiki Co., Tokyo) (6).

Decontamination tests. Decontamination activity was tested by: (i) counting the bacteria attached to the

wall, floor, or table by using the sausage agar method (9) with brain heart infusion agar slices of 10 cm^2 (four slices of sausage agar were placed in each petri dish) and (ii) evaluating the bactericidal effect on the test bacteria with *Escherichia coli* IFO3806, *Bacillus subtilis* PC1219, and *Penicillium* sp. with the same medium.

Bacteria having a concentration of 10^6/ml were placed (i) on filter paper, (ii) on filter paper under a 2-cm pad of natural fiber mattress filling, or (iii) in a 100-ml bottle of water having a diameter of 6 cm or (iv) were soaked in a rubber sponge.

Long-term ozone decontamination. The above-mentioned ozone decontamination was conducted several times in the bioclean rooms during a 14-month period, and the number of attached bacteria was checked with the sausage agar.

RESULTS

Room conditions. In the ozone 1 experiment, the room temperature was kept at an almost constant level of 24 to 26°C, and the relative humidity was kept at 49 to 50%. In the ozone 2 experiment, the temperature was maintained at 37 to 40°C, and the relative humidity was maintained at 64 to 72% by a panel heater and humidifier. In the formaldehyde experiment, the temperature was kept at 24 to 30°C, and the relative humidity was kept at 84% during formaldehyde vaporization. The humidity was gradually decreased to a rather constant level of 50% after 24 h.

Ozone and formaldehyde concentrations. The ozone concentration rose to 80 mg/m^3 after 1 h and was maintained at this level for 72 h (Fig. 1). When the ozone generator was switched off and ventilation began, the ozone concentration fell very rapidly and could no longer be detected 60 min later. Almost immediately after the start of formaldehyde vaporization, the formaldehyde gas concentration rose to 150 mg/m^3, but it gradually fell to 20 mg/m^3 after 72 h. When ventilation started, the concentration decreased slowly. The formaldehyde concentration was 6 mg/m^3 on day 4, and entry into the room even on day 7 after ventilation produced slight eye irritation.

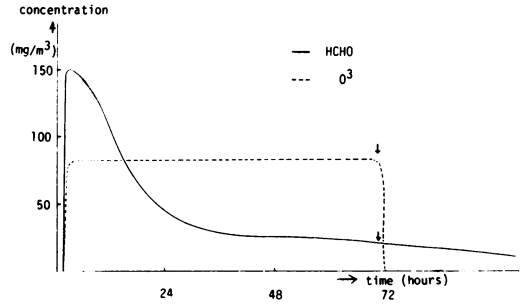

FIG. 1. Concentration of ozone (O^3) and formaldehyde (HCHO). The ozone concentration was almost constant during decontamination. The formaldehyde concentration decreased gradually.

Decontamination effects and exposure time. The relation between decontamination effects and exposure time was preliminarily examined on test organisms placed on filter paper in the same bioclean room. *E. coli* and *Penicillium* sp. were killed within 48 h, but *B. subtilis* yielded one colony, which perished within 72 h (Table 1).

Decontamination effects on test organisms. The growth of *E. coli*, *B. subtilis*, and *Penicillium* sp. on filter paper was inhibited by both ozone and formaldehyde (Table 2). No inhibition by ozone was observed for *E. coli* and *B. subtilis* on filter paper placed under a 2-cm pad on natural fiber mattress filling, but *Penicillium* sp. was inhibited, although incompletely. Formaldehyde showed a marked inhibitory effect on all three

TABLE 1. Ozone decontamination and exposure time[a]

Organism	Exposure to:	No. of colonies remaining after:		
		24 h	48 h	72 h
E. coli	Ozone	4		
		7		
	Control	1.3×10^2	8.3×10	5.8×10
			9.5×10	2.4×10
B. subtilis	Ozone	$>10^3$		
		$>10^3$	1	
	Control	$>10^3$	$>10^3$	$>10^3$
			$>10^3$	$>10^3$
Penicillium sp.	Ozone			
	Control	$>10^3$	$>10^3$	$>10^3$
			$>10^3$	$>10^3$

[a] A total of 10^6 test organisms were placed on filter paper. After decontamination, the filter paper was cultured on a plate containing brain heart infusion broth medium. Ozone concentration, 82 mg/m^3.

TABLE 2. Decontamination effect on test organisms[a]

Bacteria location	Exposure to:	No. of remaining colonies of:		
		E. coli	*B. subtilis*	*Penicillium* sp.
Filter paper	Ozone			
	Formaldehyde			
	Control	1.0×10^2	$>10^3$	$>10^3$
Filter paper under mattress filling	Ozone	7.9×10	$>10^3$	1.5
	Formaldehyde			
	Control	1.1×10^2	$>10^3$	$>10^3$
Water	Ozone		7.0	0.5
	Formaldehyde		1.7×10^3	
	Control	$>10^4$	1.0×10^3	1.9×10
Sponge	Ozone			
	Formaldehyde			
	Control	1.6×10^3	$>10^4$	2.5×10

[a] Ozone concentration, 80 mg/m^3 for 72 h. Formaldehyde concentration, 150 to 20 mg/m^3 for 72 h.

organisms under these conditions. Ozone severely inhibited colony growth of *E. coli* and *Penicillium* in a 100-ml bottle of water but inhibited growth of *B. subtilis* only slightly. Formaldehyde inhibited colony growth of *E. coli* and *Penicillium* severely but had no inhibitory effect on *B. subtilis*. Ozone and formaldehyde inhibited the growth of all three organisms in water soaked in a rubber sponge.

Number of bacteria determined by sausage agar method. The number of bacteria attached to various parts of the room was determined by using 10-cm^2 samples of sausage agar placed at about 120 points in the room (Table 3). More organisms were found on the horizontal surfaces (floor, table, sink, shower, storage cart, outside floor, bed, and toilet) than on the vertical surfaces (window, wall, door, and curtain) both before and after decontamination.

After ozone decontamination, a marked decrease of bacterial colonies was observed, with a tendency for more bacteria to remain at sites where bacteria existed before decontamination.

No significant difference was observed in the number of colonies between the ozone 1 experiment and the ozone 2 experiment although there

TABLE 3. Ozone and formaldehyde decontamination of attached bacteria[a]

Location	No. of slices	No. before				No. remaining after				No. remaining after formaldehyde expt	
		Ozone 1 expt		Ozone 2 expt		Ozone 1 (1')[b] expt		Ozone 2 expt			
		Bacteria[c]	Fungus[d]	Bacteria[c]	Fungus[d]	Bacteria[c]	Fungus[d]	Bacteria[c]	Fungus[d]	Bacteria[c]	Fungus[d]
Floor	16	213	9	196	14	7 (3)	2 (1)	16	2	0	0
Window	12	1	0	0	0	0 (0)	0 (0)	0	0	0	0
Wall	12	8	0	2	1	0 (0)	0 (0)	0	0	0	0
Ceiling	8	1	1	0	0	0 (0)	0 (0)	0	0	0	0
Table	8	37	8	158	4	2 (0)	0 (0)	2	0	0	0
Sink	8	45	8	0	0	3 (2)	1 (0)	0	0	0	0
Shower	12	31	4	18	0	5 (0)	1 (0)	3	1	0	0
Door	8	4	0	10	2	2 (2)	0 (0)	2	0	5	0
Storage cart	8	81	1	53	4	11 (0)	2 (1)	11	1	1	0
Outside floor	8	71	3	126	8	15 (12)	2 (0)	22	0	6	0
Bed	8	63	2	34	4	13 (3)	0 (0)	20	0	9	1
Toilet	4	2	0	16	2	2 (0)	0 (0)	3	0	0	0
Curtain	4	0	0	6	0	0 (0)	0 (0)	0	0	0	0
Others	0–16	9	1	0	0	0 (0)	0 (0)	17	0	1	0

[a] Ozone decontamination, 80 mg/m^3 for 72 h. Formaldehyde decontamination, 150 to 20 mg/m^3 for 72 h.
[b] Numbers in parentheses indicate bacteria remaining after ozone 1' decontamination.
[c] Number of colonies tested with 10-cm^2 samples of sausage agar.
[d] Number of positive slices.

TABLE 4. Number of bacterial colonies before and after decontamination[a]

Treatment	No. of slices	No. of sausage agar slices with bacterial colony counts of:					
		0	1–3	4–9	10–20	20<	Mean ± SD
Before decontamination:							
1	120	56 (46.6)	15 (12.5)	27 (22.5)	18 (15.0)	4 (3.3)	4.72 ± 6.40
2	124	42 (33.9)	27 (21.8)	30 (24.2)	22 (17.7)	3 (2.4)	4.99 ± 6.83
After ozone expt:							
1	116	86 (74.1)	27 (23.3)	3 (2.6)	0 (0)	0 (0)	0.52 ± 1.26
1′	128	113 (88.3)	14 (10.9)	1 (0.8)	0 (0)	0 (0)	0.17 ± 0.39
2	140	93 (66.4)	37 (26.4)	10 (7.1)	0 (0)	0 (0)	0.70 ± 1.48
After formaldehyde expt	140	129 (92.1)	8 (5.7)	3 (2.1)	0 (0)	0 (0)	0.16 ± 0.78

[a] Numbers in parentheses indicate percentages. Ozone concentration: 80 mg/m^3 for 72 h. Formaldehyde concentration: 150 to 20 mg/m^3 for 72 h. Colonies were tested with 10-cm^2 sausage agar.

was a difference of 10°C and 10% in relative humidity between the two. A mean colony count of more than one was found on the storage cart, floor, shower, and bed. After formaldehyde decontamination, the mean colony count was more than one only on the bed.

The number of sausage agar slices without bacterial colonies rose from 46.6 and 33.9% before decontamination to 74.1 and 66.4% after ozone decontamination, respectively, to 88.3% after the second use of ozone and to 92.1% formaldehyde decontamination (Table 4). Mean bacterial colonies per slice decreased from 4.72 and 4.99 before decontamination to 0.52 and 0.70 after the first ozone decontamination, respectively, to 0.17 after the second ozone decontamination and to 0.16 after formaldehyde decontamination.

For the fungus, a colony count could not be precisely made because colonies were confluent over the agar slices. However, the number of slices positive for the fungus decreased from 37/120 and 39/124 before decontamination to 8/116 and 4/140 after the first ozone decontamination, respectively, to 2/128 after the second ozone decontamination and to 1/140 after formaldehyde decontamination.

Shown in Table 5 are the changes in mean bacterial colonies by the sausage agar method in the bioclean room in which ozone decontamination was performed before each admission of 11 patients from April 1978 to June 1979. The mean colony number decreased from 4.72/10 cm^2 before and 0.52/10 cm^2 after ozone decontamination in April 1978, to 0.95/10 cm^2 and 0.02/10 cm^2 after ozone decontamination in June 1979, respectively.

Caustic effects. Ozone decontamination caused marked corrosion of rubber; many cracks were noted in the rubber parts of the sphygmomanometer and even in new rubber bags. However, no change was observed in painted metals, stainless steel, vinyl, or melamine laminates. This change in the rubber parts was not observed after formaldehyde decontamination. The metallic parts of the rooms were made of stainless steel and aluminum and were not affected after either ozone or formaldehyde decontamination. Iron nails used as test samples rusted after both ozone and formaldehyde decontamination.

DISCUSSION

Many methods have been used for the decontamination of bioclean rooms, such as peracetic acid fogging (8), formaldehyde vaporization, organic tin compound (Biomet 611) (1), and chlorhexidine spray, but none of these methods is perfect. The most frequent complaints regarding these procedures are inhalation of disinfectants by the hospital staff, inconvenience, and long retention of the unpleasant odor of the disinfectants.

Ozone decontamination has been shown to have substantial advantages. It can be switched on and off from the outside after the room has been made airtight. Compared with formaldehyde, ozone showed a very rapid decrease in concentration after the ventilating fan was turned on. This in itself is a considerable advantage in the management of bioclean rooms. The difference in the rate of expulsion may be attributable to the high adsorption of formaldehyde (2)

TABLE 5. Change of mean bacterial colonies in a laminar airflow room decontaminated with ozone.[a]

Date	No. before decontamination	No. after decontamination
April 1978	4.72	0.52
August 1978	3.51	0.20
January 1979	1.82	0.08
June 1979	0.95	0.02

[a] Colonies on sausage agar slices at about 120 points were counted in the same way as in Table 3. Ozone concentration, 80 mg/m^3 for 72 h.

of various materials and to the high degradation of ozone after the start of ventilation.

Wiping with a liquid disinfectant is very simple and effective and can be done even during the patient's stay, especially if the polluted area is limited. For general decontamination before the admission of patients, wiping with a disinfectant requires a great deal of work and is unsuitable for vinyl curtains or ceilings.

Decontamination with gas or with liquid spray after routine, nonsterilized cleanup is easier than wipe-down decontamination.

Several experiments have been conducted on the decontamination effect of ozone for the control of fungi and bacteria (4) in food protection, drinking water purification, and the decontamination of contact lenses (5). According to Elford and van den Eude (3), a sterilizing effect of ozone can be observed at 20 ppm (vol/vol), and relative humidity is an important factor. Rabotnova et al. (7) have tested the biocidal activity of ozone at 150 or 5,500 ppm (vol/vol) for 10 to 30 min and have observed satisfactory biocidal effects.

In our study, ozone proved to be a good decontaminant of test organisms at 40 ppm (vol/vol) for 3 days, but it was inferior to formaldehyde in penetrating layers of natural fiber mattress filling or in decontaminating bacteria attached to the floor. Ozone decontamination decreased the number of bacteria colonies to the level of 0.5/10 cm^2 and formaldehyde to 0.16/10 cm^2, but when the ozone-decontaminated room was redecontaminated with ozone, the mean colonies could be decreased to the level of 0.17/10 cm^2.

Bodey and Gewertz (1) have reported that among the cultures of floor samples obtained from bioclean rooms by swabbing, 68% were sterile, and the average count of organisms was 150/ft^2 (1.6/10 cm^2) after fogging with an organic tin compound (Biomet 611), whereas the wall and furniture samples were all sterile.

In this study, most of the bacterial colonies were observed on horizontal surfaces, in particular on the floor, and 74.1 and 66.4% of the agar plates did not contain colonies (the average being 0.5 organism per 10 cm^2) after the first ozone decontamination. These data suggest that ozone decontamination is as effective as decontamination with Biomet 611.

Furthermore, the number of bacterial colonies decreased gradually between April 1978 and June 1979, when 11 patients were treated in the room, and before each admission ozone decontamination was performed. This also suggests that ozone is useful for decontaminating bioclean rooms.

The caustic effect of ozone upon rubber materials was more severe than expected. This indicates the necessity of selecting materials resistant to ozone when bioclean rooms are constructed. The rubber materials in our rooms were replaced with glass wool and vinyl plastics so that the ozone decontamination could be made without any severe effects on the facilities.

In conclusion, although ozone decontamination has poorer penetration activity and greater caustic effects than does formaldehyde, it is much superior to formaldehyde with regard to convenience, ready expulsion after use, and the insignificant inhalation of the disinfectant by the hospital staff. Because the disadvantages can be adequately controlled if proper consideration is made in selecting the materials used in constructing the bioclean rooms and in sterilizing mattresses and linen with ethylene dioxide, the results of the present study suggest that ozone is useful for the decontamination of bioclean rooms.

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LITERATURE CITED

1. **Bodey, G. P., and B. Gewertz.** 1969. Microbiological studies of a laminar air flow unit for patients. Arch. Environ. Health **19**:798–805.
2. **Braswell, J. R., D. R. Spiner, and R. K. Hoffman.** 1970. Adsorption of formaldehyde by various surfaces during gaseous decontamination. Appl. Microbiol. **20**:765–769.
3. **Elford, W. J., and J. van den Eude.** 1942. An investigation of the merits of ozone as an aerial disinfectant. J. Hyg. **42**:240–265.
4. **Friedlander, S. K. (chairman), Committee on Medical and Biologic Effects of Environmental Pollutants.** 1977. Ozone and other photochemical oxidants, p. 545–547. National Academy of Sciences, Washington, D.C.
5. **Kamiki, T., and Y. Kikkawa.** 1976. Ozone sterilization technique of hydrophilic contact lenses. Contacto Int. Contact Lenses J. **20**:16–18.
6. **Pharmaceutical Society of Japan.** 1973. Standard methods of analysis for hygenic chemists, p. 940 and 1067. Kanahara Press, Tokyo.
7. **Rabotnova, I. L., V. S. Somov, T. S. Bobkova, I. V. Zlochevskaya, L. N. Chekunova, I. F. Knyazeva, and S. I. Belen'kiy.** 1971. The relationship between the toxic action of ozone on yeast and certain components and medium pH. Vestn. Mosk. Univ. Biol. Pochvoved. **6**:47–51, (In Russian.)
8. **Schwartz, S. A., and S. Perry.** 1966. Patient protection in cancer chemotherapy. J. Am. Med. Assoc. **197**:623–627.
9. **Ten, C. L.** 1965. A note on a simple method of bacteriological sampling by means of agar sausages. J. Appl. Bacteriol. **28**:221–223.