industries. Dust respirators are normally employed wherever abnormal concentrations of dusts are encountered, such as in coal mines. These respirators have a very low resistance to breathing and could be readily used in hospitals and medical or biological laboratories.

ACKNOWLEDGMENTS

The authors are indebted to Fred E. White and William V. McKnight, HM2, USN, for technical assistance.

SUMMARY

The method used in evaluating the respiratory protection provided by representative types of contagion masks against an aerosol of *Bacillus subtilis* var. *niger* spores has been described.

It can be concluded that the contagion masks evaluated offer poor respiratory protection against airborne microorganisms in the particle size range of 1.0 to 5.0 μ in diameter.

Commercially available dust respirators and related masks are approximately 2.5 to 5 times more efficient than contagion masks. An even higher degree of protection can be obtained by the use of full-face masks.

REFERENCES

GUYTON, H. G., AND LENSE, F. T. 1955 Methods for evaluating respiratory protective masks and their component parts. Paper presented at 127th meeting of the American Chemical Society.

SONKIN, L. S. 1950 Application of the cascade impactor to studies of bacterial aerosols. Am. J. Hyg., **51**, 319–342.

Relative Resistances of Microorganisms to Cathode Rays

I. Nonsporeforming Bacteria

WON YOUNG KOH, CATHERINE T. MOREHOUSE AND VELMA L. CHANDLER

Department of Microbiology, Research Division, Ethicon, Inc., New Brunswick, New Jersey

Received for publication January 12, 1956

Doses of cathode rays required to inhibit microorganisms, under various conditions of test, have been reported by others. Dunn *et al.* (1948) irradiated *Micrococcus pyogenes* var. *aureus* (*Staphylococcus aureus*), *Escherichia coli*, *Serratia marcescens*, a sporeforming bacillus, yeasts and a fungus. Dunn (1952) reported doses required to kill 30 species including bacteria, yeasts and fungi. Katznelson and his associates (1952) studied the quantitative relationship between number of organisms and dose required for sterilization of honey. Studies with a common contaminant, *Micrococcus pyogenes* var. *aureus* (*S. aureus*), were reported by Bellamy and Lawton (1954). The relative resistances of yeasts and molds (Bridges *et al.*, 1956) and bacterial spores (Pepper *et al.*, 1956) to cathode rays have been reported from our laboratories.

In view of the few data available, it was deemed advisable to establish relative resistances for a broad spectrum of nonsporeforming organisms to cathode radiation. Differences in resistances between 24-hr and 5- to 7-day cultures of several species were also determined.

It is well known that following ionizing radiation the survival of a mass population is exponential (Lea, 1946; Bacq and Alexander, 1955). This fact suggested that the sterilizing[1] dose may be dependent upon the concentration of cells. Therefore the relationships between sterilizing dose and cell concentration as well as sterilizing dose and volume of the suspension were investigated. Since it had been observed that more concentrated suspensions required higher sterilizing doses, it was also of interest to determine the limits of this correlation.

MATERIALS AND METHODS

The test organisms were various species of nonsporeforming bacteria which have been maintained in our laboratory for several years. For these experiments, organisms were grown in 10 ml of their optimum liquid media. The majority of the cultures were incubated at 37 C (*Leuconostoc* at 25 C) for 18 to 24 hr. Pneumococci, streptococci, micrococci and sarcina required 48-hour incubation for maximum growth. A 10-day culture of *Mycobacterium tuberculosis* was employed. After incubation, cells were washed twice by centrifugation and resuspended in distilled water to the original volume. Prestandardization of numbers of viable cells per a unit volume of suspension proved extremely difficult and was not attempted. When possible, the number of viable

[1] The term "sterility" here denotes inability of the microorganisms to reproduce under these conditions of test.

cells in the prepared suspension was determined for comparison and interpretation of experimental results.

To study the relative resistances of bacteria, sterilized filter paper discs[2] were saturated with 0.1-ml aliquots of a suspension. Triplicate discs for each organism-dose were placed in double polyethylene envelopes[3] and hermetically sealed. The envelopes were then exposed to cathode rays. After irradiation the outside envelopes were chemically disinfected and the discs aseptically transferred to recovery media. The recovery media employed were those known to be optimal for growth of the organisms. The majority of tests were incubated for 21 days at 37 C; *Leuconostoc* was incubated at 25 C.

In another series of experiments, a special medium (Krask, 1953) was employed to inhibit production of bacterial spores in order to compare the resistances of vegetative cells of two organisms of the *Bacillus* genus with their respective spores.

For studies dealing with the relationships between sterilizing dose and cell concentration and between sterilizing dose and volume, bacterial and spore suspensions were put into glass tubes[4] and the tubes sealed. The tubes were irradiated with various doses and the contents tested for sterility.

For the studies of the radiation doses required to sterilize concentrated bacterial suspensions, 0.1-ml aliquots of serially diluted suspensions of washed *E. coli* cells were streaked onto agar (2 per cent Bacto agar in distilled water) plates. This procedure provided a thin layer of inoculum. After irradiation of the open plates at various doses, a thin layer of nutrient agar was poured over the surface. Colonies of survivors, which appeared between the two agar layers following incubation for 48 hr at 37 C, were counted and survival curves drawn.

A two million electron volt Van de Graaff accelerator[5] was employed as the source of cathode rays.

Results and Discussion

Relative resistances. The resistances of a group of gram positive bacteria are shown in table 1. In general, gram negative bacteria were less resistant than gram positive bacteria, as shown in table 2.

Some types of *Diplococcus pneumoniae* were found to be the most resistant of the nonsporeforming bacteria. Other encapsulated organisms failed to exhibit the same degree of resistance. This observation would indicate that the conspicuously high resistance of *D. pneumoniae* among nonsporeforming bacteria is not due to the protection rendered by the rich capsule material.

Lea *et al.* (1937) interpreted the killing of bacteria as a "lethal mutation, produced by a single ionization within

Table 1. *Resistances of gram positive bacteria*

	Organism	Cells/ml	Highest Dose Showing All Positive Cultures	Lowest Dose Showing All Negative Cultures
			*megarep**	*megarep*
E-12	*Micrococcus pyogenes* var. *aureus*	1×10^8	0.05	0.15
E-20	*Alcaligenes ammoniagenes*	2.7×10^6	0.125	0.15
E-54	*Diplococcus pneumoniae* (I)		0.3	0.4
E-55	*Diplococcus pneumoniae* (III)		0.4	0.5
E-21	*Sarcina lutea*	$>1 \times 10^4$	0.1	0.4
E-13	*Micrococcus pyogenes* var. *albus*		0.025	0.05
E-14	*Micrococcus conglomeratus*		0.2	—
E-4	*Corynebacterium acnes*	2.5×10^6	0.1	0.2
E-15	*Micrococcus pyogenes* var. *aureus* (hemolyticus)		0.05	0.15
E-17	*Streptococcus* sp. (Lancefield hemo. streptococcus)		0.15	0.3
E-29	*Streptococcus pyogenes*	9.2×10^5	0.15	0.3
E-36	*Corynebacterium acnes*		0.1	0.2
E-102	*Diplococcus pneumoniae* (I)		0.3	0.4
E-103	*Diplococcus pneumoniae* (II)		0.25	0.4
E-104	*Diplococcus pneumoniae* (III)		0.4	0.45
E-105	*Diplococcus pneumoniae* (IV)	1.6×10^8	0.3	0.45
E-61	*Leuconostoc mesenteroides*	3.9×10^7	—	0.1
E-64	*Leuconostoc mesenteroides*	5.2×10^6	—	0.1
E-67	*Leuconostoc dextranicum*	9×10^6	—	0.1
E-79	*Leuconostoc dextranicum*	3.4×10^8	—	0.1
E-71	*Alcaligenes viscosus*		—	0.1

* Megarep = 1 million reps (Roentgen equivalent physical units).

Table 2. *Resistances of gram negative bacteria*

	Organism	Cells/ml	Highest Dose Showing All Positive Cultures	Lowest Dose Showing All Negative Cultures
			*megarep**	*megarep*
E-38	*Escherichia coli*	2×10^8	0.05	0.075
E-39	*Pseudomonas aeruginosa*	1.26×10^8	—	0.025
E-24	*Proteus vulgaris*		0.025	0.050
E-28	*Salmonella typhosa*		—	0.025
E-35	*Salmonella schottmuelleri*		0.075	0.1
E-63	*Serratia marcesens*		—	0.05
E-1	*Escherichia coli*		0.05	0.15
E-18	*Pseudomonas aeruginosa*	2×10^8	—	0.25
E-26	*Klebsiella pneumoniae*	1.1×10^8	0.1	0.2
E-87	*Klebsiella pneumoniae*	4×10^8	0.075	0.15
E-107	*Aerobacter aerogenes*	3.25×10^8	—	0.05
E-82	*Bacteroides vulgatus*		—	0.025
E-83	*Bacteroides ovatus*		—	0.025

* 1 Megarep = 1 million reps.

[2] Schleicher & Schuell Co., Keene, New Hampshire. No. 740-E, diameter 12.7 mm.

[3] Fabricated from commercially available polyethylene tubing with a wall thickness of 5 mil.

[4] Sodium glass O.D. 11 mm, length 100 mm and wall thickness 1 mm.

[5] Built by High Voltage Engineering Corporation, Cambridge, Massachusetts.

a sensitive volume," which they tentatively identified with chromosomal material. According to their view, the exceptionally high resistance of *D. pneumoniae* could be explained by assuming an unusually small physical size of chromosomes or genes.

It will be seen in table 3 that bacterial spores of a given species are more resistant to cathode rays than are their respective vegetative cells.

The effect of age of cells on resistance to radiation of a number of cultures of various species was studied. Three representative studies are shown in table 4. It will be noted that the cells from old cultures were less resistant than were cells from young cultures. Fewer viable cells were present in the older preparations. It is difficult to determine from these data whether age of cells or differences in number of viable cells was responsible for the differences in doses required to sterilize.

Sterilizing dose as a function of cell concentration and volume of cell suspension. The results of two experiments employing *E. coli* and *Clostridium sporogenes* spores are shown in figure 1. In both instances, when the volume of suspension remained constant and the concentration of bacteria or spores increased, higher doses were required to prevent multiplication. When the cell concentration remained constant, a larger volume required an appreciably higher dose to sterilize than did a smaller volume. Similar results were obtained with the spores of *Bacillus subtilis*, *Bacillus mesentericus* and *Clostridium tetani*.

TABLE 3. *Relative resistances of vegetative cells and spores of same organism*

Organism	Cells/ml	Highest Dose Showing All Positive Cultures	Lowest Dose Showing All Negative Cultures
		*megarep**	*megarep*
Bacillus mesentericus			
Vegetative cells	2.1×10^4	—	0.3
Spores	5×10^5	1.3	1.8
Bacillus species (E-594)			
Vegetative cells	1.9×10^5	0.05	0.5
Spores	5×10^5	1.7	2.0

* 1 Megarep = 1 million reps.

TABLE 4. *Resistance as a function of culture age and cell concentration*

Organism	Cells/ml	Highest Dose Showing All Positive Cultures	Lowest Dose Showing All Negative Cultures
		*megarep**	*megarep*
Streptococcus pyogenes (hemolytic)			
24 hr	9.2×10^8	0.15	0.3
7 days	3.7×10^5	0.05	0.16
Diplococcus pneumoniae (IV)			
24 hr	1.6×10^8	0.3	0.45
5 days	1.2×10^5	—	0.1
Leuconostoc dextranicum			
24 hr	3.4×10^8	—	0.1
7 days	2.7×10^7	0.025	0.05

* 1 Megarep = 1 million reps.

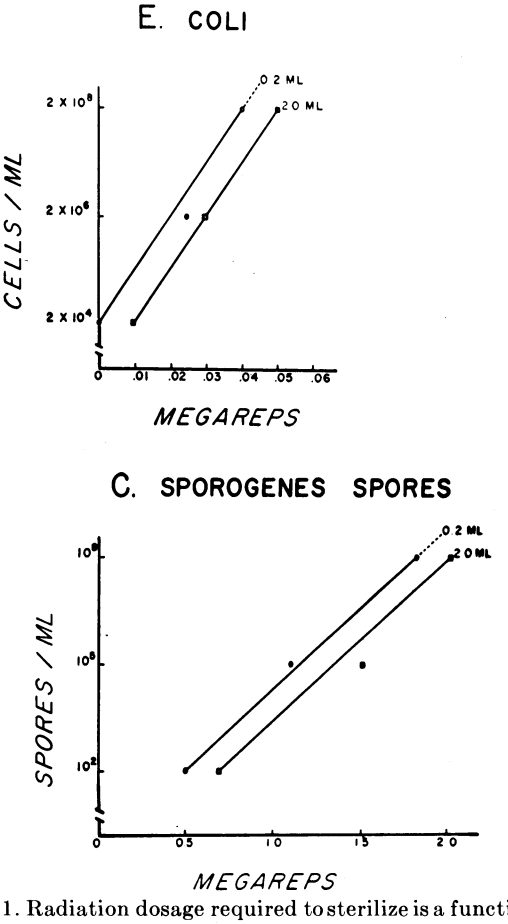

FIG. 1. Radiation dosage required to sterilize is a function of cell concentration and volume of suspension.

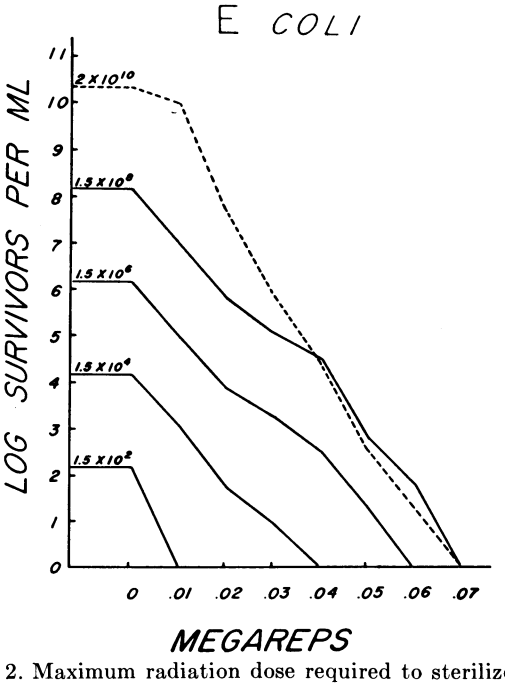

FIG. 2. Maximum radiation dose required to sterilize irrespective of increased concentration of cells.

Limits of sterilizing dose as a function of cell concentration. When the cells of *E. coli* were irradiated in various concentrations on agar plates, the survival patterns shown in figure 2 were obtained. A highly concentrated suspension provided a cell count of 2×10^{-10}/ml. At a higher concentration, the suspension was too thick to permit pipetting. The survival rate in the highly concentrated sample decreased sharply as the dose increased. It appears that when the cell concentration exceeded a certain limit no greater doses were required to attain sterility.

ACKNOWLEDGMENTS

The technical assistance rendered by Mr. John R. Tekowitz and by the Department of Pharmaceutical Chemistry is acknowledged.

SUMMARY

Representative species of nonsporeforming bacteria were exposed to cathode rays generated by a two million electron volt (MEV) Van de Graaff accelerator. The doses required to effect sterility of various organisms under these conditions of test are reported. All nonsporeforming bacteria tested were susceptible to doses of 0.5 megarep or less. Gram negative bacteria were more sensitive to irradiation than gram positive. The most resistant species tested was *Diplococcus pneumoniae*. Vegetative cells of bacterial sporeformers were more sensitive than their respective spores.

When the volume of suspension was constant, an increase in concentration of cells or spores required higher radiation doses for sterilization. When the volume was increased and the concentration of cells or spores remained constant, a higher dose was also required. When the cell concentration of *Escherichia coli* exceeded certain limits, no greater doses were required to attain sterility.

REFERENCES

BACQ, Z. M., AND ALEXANDER, P. 1955 *Fundamentals of radiobiology*, 1st ed. Academic Press Inc., New York.

BELLAMY, W. C., AND LAWTON, E. J. 1954 Problems in using high-voltage electrons for sterilization. Nucleonics, **12**, 54–57.

BRIDGES, A. E., OLIVO, J. P., AND CHANDLER, V. L. 1956 Relative resistances of microorganisms to cathode rays. II. Yeasts and molds. Appl. Microbiol., **4**, 147–149.

DUNN, C. G., CAMPBELL, W. L., FRAM, H., AND HUTCHINS, A. 1948 Biological and photo-chemical effects of high energy, electrostatically produced Roentgen rays and cathode rays. J. Appl. Phys., **19**, 605–616.

DUNN, C. G. 1952 Microbiological aspects of ionizing radiations as a means of sterilization. Abstract prepared for Industrial Liaison Conference on Food Sterilization. Dept. Food Technol., Massachusetts Inst. Technol., Cambridge, Massachusetts.

KATZNELSON, H., JAMIESON, C. A., LAWTON, E. J., AND BELLAMY, W. D. 1952 Studies on the treatment of contaminated combs and honey with high velocity electrons. Can. J. Technol., **30**, 95–103.

KRASK, B. J. 1953 Methionine sulfoxide and specific inhibition of sporulation in *Bacillus subtilis*. J. Bacteriol., **66**, 374.

LEA, D. E., HAINES, R. B., AND COULSON, C. A. 1937 Actions of γ-rays on bacteria. Proc. Roy. Soc., B 123, 1.

LEA, D. E. 1946 *Actions of Radiation on Living Cells*, 1st ed. Cambridge University Press, New York.

PEPPER, R. E., BUFFA, N. T., AND CHANDLER, V. L. 1956 Relative resistances of microorganisms to cathode rays. III. Bacterial spores. Appl. microbiol., **4**, 149–152.