Radiation sterilization

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SYNOPSIS The sterilization by radiation of disposable medical items has been investigated. Various vegetative and spore-bearing bacteria have been irradiated and results indicate that 2.5 Mrad can be recommended to give a high degree of sterility. Spores of *B. pumilus* (E.601) showed such resistance that it might be a suitable test organism for determining the efficiency of a radiation treatment. A high standard of packaging must be demanded for pre-sterilized products.

Several pre-sterilized disposable medical items, such as plastic hypodermic syringes and catheters, are now available in Great Britain and many more are likely to be marketed in the near future. It is often necessary for economic reasons to manufacture some of these items from cheap thermoplastic materials so that a 'cold' method of sterilization must be used for treating appliances made in this way.

Two main sterilizing agents are available to the manufacturer, ethylene oxide and ionizing radiations, and the final choice of technique is determined by the particular application. Sterilization by radiation is achieved in practice by the use of either high-energy electrons from a machine, such as a linear accelerator, or gamma radiation from an isotope source such as cobalt 60. Both types of ionizing radiation have been used commercially for sterilization, and they have similar bactericidal properties, but technically they differ in the characteristics of dose rate and degree of penetration. Absorbable surgical sutures sterilized by high-energy electrons are marketed in the United States by Ethicon Incorporated (Artandi and Van Winkle, 1959) and sutures are also being sterilized by gamma radiation in Melbourne, Australia, at a large cobalt 60 installation which is basically intended for treating imported animal fibres contaminated by anthrax. The package irradiation plant at the Wantage Research Laboratory is now being used to sterilize large quantities of medical products intended for industry and hospitals for marketing and clinical trials. Several items sterilized at this plant are now available on a limited scale in this country. Radiation sterilization is not, however, the answer to all sterilization problems because certain products are adversely affected by the treatment. Nevertheless, experience in the past few years has shown that it is a very useful addition to the sterilization techniques

available, in particular to the manufacturers of medical and pharmaceutical products. The current status of radiation sterilization has recently been reviewed by Powell and Bridges (1960).

The purpose of this paper is to report the bacteriological results and some practical experiences of two laboratories concerned with the application of radiation sterilization. Part I deals with application to disposable balloon catheters and Part II concerns treatment of disposable plastic hypodermic syringes.

STERILIZATION OF CATHETERS

METHOD The following spore-bearing organisms were used in the trials and were prepared as previously described (Darmady, Hughes, and Jones, 1958): *B. stearothermophilus*, N.C.I.B. No. 8157, *Cl. tetani*, N.C.T.C. No. 5411, and dried garden soil.

From microscope slides small rectangular glass strips about 26 × 5 mm. were cut. On to these were placed either two drops of a broth suspension of *B. stearothermophilus* spores, two drops of a Robertson's meat broth culture of *Cl. tetani* containing at least 50% spores, or two drops of a suspension of dried soil in serum. The slides were dried in air at a temperature of 60°C. for 30 minutes and placed in lightly plugged 3 in. × ½ in. pyrex tubes. The material was sent to Wantage. It was irradiated by a cobalt 60 source and on return to Portsmouth the organisms were tested for viability by the following methods:

B. stearothermophilus, by dropping the slide into a tube of broth and incubating at 60°C. for 48 hours.

Cl. tetani, by dropping the slide into a tube of Robertson's meat medium and incubating at 37°C. for seven days, and then subculturing on to blood agar under anaerobic conditions, readings being made at 48 hours and seven days.

Dried soil, by dropping half the total number of slides into nutrient broth and half into Robertson's meat

medium. After seven days' incubation subcultures were made on to blood agar aerobically from the former and anaerobically from the latter. Readings were again made at 48 hours and seven days.

On return from Wantage about 10% of the slides from each experiment were stored aseptically in daylight at room temperature for 28 days before being cultured to guard against the possibility of the organisms regenerating.

In all cases control slides were kept in the laboratory at room temperature until the test slides had been received back from Wantage. They were then treated in a similar fashion.

Approximately 100 slides of each organism were subjected to each dose of radiation and the results are shown in Table II.

Several catheters were infected in a similar way with *Cl. tetani* and dried soil and irradiated at various dose levels. The numbers tested are shown in Table I. They were tested for sterility using the techniques described above.

TABLE I

RESULTS OF STERILITY TESTS ON INFECTED CATHETERS

Organisms Tested	Numbers of Catheters Examined		
	2.0 Mrad	2.5 Mrad	3.0 Mrad
<i>Cl. tetani</i>	38	6	2
Dried soil	6	6	2

RESULTS Growth occurred in all controls retained in the laboratory. None of the organisms tested survived a dose of 2.0 Mrad and there was no evidence of photoregeneration after exposure to daylight at room temperature for 28 days. *Cl. tetani* appeared to be the most resistant of the organisms tested. All infected catheters were found to be sterile at all dose levels.

TABLE II

RESULTS OF STERILITY TESTS ON IRRADIATED SAMPLES OF GLASS SLIDES

Organisms Tested	Percentage Sterile		
	1.0 Mrad	1.5 Mrad	2.0 Mrad
<i>B. stearothermophilus</i> (a)	90 (90)	100 (80)	100 (90)
(b)	90 (10)	100 (10)	100 (10)
<i>Cl. tetani</i> (a)	83 (80)	92 (90)	100 (90)
(b)	100 (10)	90 (10)	100 (10)
Dried soil (a)	95 (90)	100 (90)	100 (94)
(b)	100 (10)	100 (10)	100 (10)

Figures in brackets denote number of slides tested; (a) on return from Wantage and (b) after photoregeneration test.

STERILIZATION OF PLASTIC HYPODERMIC SYRINGES

METHOD Twelve organisms, six vegetative and six spore-bearing, were used for this investigation.

The vegetative organisms were grown in nutrient or glucose nutrient broth at optimum temperature for 18 hours. The spore-bearers, after specific spore preparation, were used as suspensions in sterile distilled water.

PREPARATION OF SPORES For *B. subtilis* and *B. pumilus*, an overnight nutrient broth culture was flooded on the dried surface of a nutrient agar plate, incubated 18 hours at 37°C., then left for two days at room temperature.

For *B. globigii*, an overnight glucose nutrient broth culture was flooded on the dried surface of glucose plus 1% activated charcoal agar plate, incubated 18 hours at 37°C., then left for four days at room temperature.

For *B. stearothermophilus*, as recommended by Alder and Gillespie (1957).

For *Cl. welchii*, as described by Ellner (1956).

For *Cl. tetani*, a Robertson's meat broth culture was incubated for three days at 37°C., then left at room temperature until good sporulation occurred.

After harvesting and washing with sterile distilled water, the spore suspensions were heated at 80°C. for 10 minutes except for *B. stearothermophilus* which was heated in a boiling water bath for 15 minutes.

These broth or water suspensions were used to inoculate sterile filter-paper discs by placing one drop (0.02 ml.) from a calibrated Pasteur pipette on to each disc. After drying at 55°C. for 30 minutes, each disc was placed aseptically in a small polystyrene container with a polyethylene cap and sealed in a polyethylene envelope, simulating materials used in the manufacture of plastic syringes. The packages were irradiated in the spent fuel rod assembly at A.E.R.E., Harwell.

For each of the 12 organisms tested, 100 inoculated discs were irradiated at each of four dose levels and 10 discs were kept as controls.

A surface plate count was made of one drop of each bacterial suspension, to ascertain the size of the disc inoculum.

After irradiation, the polyethylene bags and polystyrene containers were opened and each disc transferred aseptically to the appropriate liquid medium which was incubated at optimum temperature for 21 days. For these viability tests, nutrient or glucose nutrient broth was used for the vegetative organisms. For *B. subtilis* and *B. pumilus* nutrient broth, for *B. globigii* glucose nutrient broth, for *B. stearothermophilus* dextrose-tryptone broth, for *Cl. welchii* Brewer's medium, and for *Cl. tetani* Robertson's cooked meat medium were used. All bottles of media containing discs were examined after 24 hours', three days', and 21 days' incubation, when those showing growth were checked by subculturing on 10% horse blood agar. All bottles of Brewer's and Robertson's cooked meat media were subcultured on 10% horse blood agar at the end of the incubation period of 21 days. The results of this test are shown in Table III.

After this investigation a small preliminary experiment was carried out on commercially packed plastic hypodermic syringes. Fifty assembled syringes packed in a single plastic envelope were received from the manufacturers. Forty of these were irradiated at 2.5 Mrad and 10 were left unirradiated for use as controls. Using aseptic precautions to remove them from the envelope, each barrel and plunger was dropped separately into 100 ml. quantities of sterile nutrient broth and incubated at 37°C. for 21 days. After incubation, the presence of turbidity in the broth was taken as evidence of growth.

TABLE III

RESULTS OF STERILITY TESTS ON IRRADIATED SAMPLES OF INOCULATED PAPER DISCS IN PLASTIC CONTAINERS

Organisms Tested	Number of Organisms per Disc	Percentage Sterile after 21 Days' Incubation				
		Control	1.0 Mrad	1.5 Mrad	2.0 Mrad	2.5 Mrad
<i>Pseudomonas pyocyanea</i> (NMH)	2.6×10^7	0	100	100	100	100
<i>Staphylococcus aureus</i> (NMH)	1.1×10^7	0	100	100	100	100
<i>Streptococcus viridans</i> (NMH)	1.2×10^7	0	100	100	100	100
<i>Streptococcus faecalis</i> (NMH)	1.5×10^7	0	100	100	100	100
<i>Escherichia coli</i> (NMH)	8.7×10^6	0	100	100	100	100
<i>Micrococcus cryophilus</i>	4.2×10^5	0	100	100	100	100
<i>Bacillus subtilis</i> (NMH)	8.1×10^4	0	29	100	100	100
<i>Bacillus globigii</i>	1.7×10^4	0	100	100	100	100
<i>Bacillus pumilus</i> (E.601)	5.8×10^7	0	72	97	94	100
<i>Bacillus stearothermophilus</i> (8157)	1.6×10^6	0	90	100	100	100
<i>Clostridium welchii</i> (8797)	2.3×10^5	0	0	68	100	100
<i>Clostridium tetani</i> (5411)	3.2×10^5	0	100	100	100	100

One hundred paper discs per organism were irradiated at each dose level. (NMH) isolated at North Middlesex Hospital.

RESULTS. Of the six vegetative organisms tested all the inoculated discs were sterile at each of the four dose levels used. With the six spore preparations tested a greater resistance to radiation was shown by survival at the lower dose levels. Of the aerobic spores, those of *B. pumilus* were the most resistant, a few surviving after 2.0 Mrad irradiation. *Cl. welchii* was shown to be the more resistant of the two anaerobic spores tested, some surviving after 1.5 Mrad irradiation.

Of the commercially packed syringes examined after irradiation growth occurred only in one tube of broth containing a plunger. This was identified as a pure culture of a micrococcus. All the unirradiated control syringes produced heavy turbidity in broth from which spore-bearing organisms were isolated. It is suggested that the isolation of the micrococcus from one irradiated sample might indicate contamination during transfer of the plunger to the broth.

DISCUSSION

In a previous communication (Darmady, Hughes, Jones, and Verdon, 1959) it was shown that between 9% and 50% of equipment used in hospital wards and stated to be sterile was, in fact, contaminated. At that time the alleged sterility of catheters was not investigated but there was no reason to expect that the results would be any better. In order to confirm this we have recently carried out an examination of catheters prepared by traditional ward procedures and have found that out of 10 examined six were sterile and four were infected. Micrococci were isolated from all four of the infected catheters and in addition coliform bacilli were isolated from one.

It is recognized that steam under pressure will sterilize providing the catheters are properly cleaned.

In our experience this method is time consuming, and repeated heat sterilizing also causes deterioration of the rubber. In the Portsmouth laboratory other methods of treating catheters have been tried, such as formalin vapour, ethylene oxide, and soaking in various disinfectant fluids, but these have proved to be unreliable and will be the subject of a further communication. As an alternative to these methods, it seemed that radiation sterilization should be investigated for such items as catheters and disposable plastic syringes.

The results of this investigation indicate that spore-bearing organisms are more resistant to radiation than the vegetative forms, and of the organisms tested, the spores of *B. pumilus* (E.601) appear to be the most resistant. It is not considered that the higher number of spores per disc used for this organism (see Table III) could completely account for this apparent resistance as compared with the other spore-bearers. This strain has previously been examined for radiation resistance by Pepper, Buffa, and Chandler (1956) and it appears to be a possible test organism for assessing the efficiency of radiation sterilization. It requires no special spore preparation as it sporulates readily on nutrient agar. A disadvantage may be that it is difficult to differentiate between this strain and normal laboratory contaminants. Nevertheless, other bacilli used as test organisms, for example, *B. globigii* for ethylene oxide and *B. stearothermophilus* for moist heat, are not suitable for use with radiation sterilization because of their lower radiation resistance, and the clostridia, although showing some resistance to radiation, have such cultural requirements that they are unsuitable as routine test organisms.

It is apparent from the examinations of the two laboratories that there is some slight difference in the results that were obtained with *Cl. tetani*. This

difference might be due to variations in the preparation of the spores, initial concentration of spores used, the use of different supporting media, *i.e.*, glass slides and paper discs, or even due to the fact that in one laboratory the spores were not washed, so that a certain amount of broth might adhere to the spore coat, providing some degree of protection. It is not certain which of these variables can account for this difference, but these are being further investigated. Nevertheless, the results indicate that a dose of 2.5 Mrad is adequate for providing complete safety and is to be recommended. This agrees with the conclusions of other workers (Artandi and Van Winkle, 1959; Report of Association of British Pharmaceutical Industry, 1960; Oliver and Tomlinson, *in press*).

Recently, such disposable items as catheters, needles, and plastic syringes, which have been sterilized by gamma radiation at 2.5 Mrad, have become commercially available in this country. Thirty of these catheters, which were doubly packed in polyethylene envelopes, have been tested and all were sterile.

Although this method allows considerable choice of packaging materials, it is not enough to choose a material that is strong and is impermeable to micro-organisms. The standard of sealing the package must be high, and methods for aseptically removing the sterile article from the package must, wherever possible, be incorporated into the package design. As an example of inadequate sealing, we have examined over 1,600 presterilized disposable needles which were packed in polyethylene envelopes, and found that 18% of these had defective seals. Similar-

ly, we have tested the ability to remove the needle from the container and found that out of two series of needles with 400 needles in each series from two different manufacturers, 7% and 1.5% were contaminated. In each case the method of opening was to burst the hub of the needle through the packaging material, and it is clear that the needle could not be transferred to the syringe without the risk of contamination. As a control, 100 needles sterilized by dry heat in a convenient container were found to be sterile when removed.

From our observations, therefore, it is important to realize that maintenance of sterility must be beyond reproach, and it is known that many manufacturers are giving considerable thought to improving standards of packaging.

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