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The sterilization of surgical rubber gloves and plastic tubing by means of ionizing radiation

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INTRODUCTION

There is general agreement that rubber gloves are difficult to sterilize, and the position as summarized by the Central Pathology Committee of the Ministry of Health (Report, 1954) is: 'If gloves are sterilized at high temperatures (115°–120° C.) they quickly become vulcanized and useless; they are best treated in a small jacketed autoclave at 5 lb. pressure (109° C.) for 30–40 min. and then dried for 20 min. Such treatment by itself does not ensure complete sterility....' Bowie (1957) recommends sterilization at 15–17 lb./sq.in. for 15 min., which is sufficient to kill resistant spores, but he does not state how rapidly gloves deteriorate under this treatment.

Small-bore plastic tubes are likewise difficult to sterilize by heat because, even though the material may not melt, it does soften and the tubing is liable to become permanently kinked or flattened. In some applications, when joined to metal needles, plastic tubing may split owing to the different expansions which occur.

With materials, such as rubber gloves and plastic tubing, therefore, conventional heat sterilization methods are not entirely satisfactory, and the use of ionizing radiation appears to be worth consideration. This allows sterilization to be carried out without heat and through a sealed pack. As these particular items are neither thick nor bulky it is possible to consider the use of sources of either gamma radiation (^{60}Co or pile fuel rods as used at the Atomic Energy Research Establishment Laboratories, Wantage) or high energy electrons from some form of electron accelerator (Foster, Dewey & Gale, 1953; Miller, 1955) although, where metal needles are present, gamma radiation methods are probably preferable.

When bacteria or bacterial spores are exposed to ionizing radiation the logarithm of the number surviving is inversely proportional to the dose of radiation. Edwards, Peterson & Cummings (1954) found that 1 Mrep. of cathode rays reduced the numbers of viable *Bacillus subtilis* spores by a factor of 10^8. Fuld, Proctor & Goldblith (1957), however, found their strain of *B. subtilis* more sensitive. The viable count decreased by 10^7 with a dose of only 0·13 Mrep. On the other hand, a strain of *Clostridium sporogenes* was much more resistant, a reduction of only 10^5 being produced by 1·9 Mrep. Different strains of the same species of bacteria differ in their sensitivity to radiation even when tested by the same technique (Pepper, Buffa & Chandler, 1956).

Experiments have been carried out to determine the radiation dose necessary to sterilize dry spores inside rubber gloves and plastic tubing and also to study the radiation damage produced, as it was appreciated that this would, in some instances, limit the application of this method.

MATERIALS AND METHODS

Organisms and methods of culture

The organisms used were *Bacillus globigii* (*B. subtilis*), *B. stearothermophilus*, *Clostridium tetani* N.C.T.C. no. 5411, and a strain of *Cl. sporogenes* kindly supplied by Dr R. L. Vollum. The strain of *B. globigii* was grown in agitated broth culture at 30° C. for 4 days and the thermophile in a peptone medium for 4 days at 52° C. *Cl. sporogenes* was grown in Robertson's cooked meat medium; *Cl. tetani* was grown in 'exhausted' Robertson's cooked meat medium (Darmady, Hughes & Jones, 1958). After 5 days' incubation the cultures of clostridia were filtered through coarse paper. The spores were harvested by centrifugation, washed and suspended in distilled water. Samples of the spore suspensions were heated to 80° C. for 20 min., diluted appropriately and plate counts were carried out for *B. globigii*, and liquid dilution counts, in tubes of glucose broth, for *B. stearothermophilus*, and in cooked meat medium for the two clostridia.

Bundles of white cotton thread were soaked in a suspension of spores in normal horse serum and dried over calcium chloride at 4° C. Bundles of twenty $\frac{1}{2}$ in. lengths of thread were used as test objects, and from the spore count in the suspension and the known fluid uptake of the threads the approximate number of spores per thread was calculated. After irradiation each thread was cultured separately, those carrying *B. globigii* in peptone water, those with clostridia in Robertson's cooked meat at 37° C., and those with *B. stearothermophilus* in glucose broth at 52° C.

To investigate the penetration of radiation through needles, 2 in. lengths of thread infected with tetanus spores were inserted into the lumen of serum needles (19 s.w.g.) and the excess thread coiled inside the Luer mount. Pairs of such needles were exposed to each dose of radiation.

Polythene and nylon tubing with needles and syringe adaptors attached were flushed through with blood containing 10^6 tetanus spores/ml., roughly drained, dried under vacuum and exposed to various doses of radiation. After irradiation the assemblies were cut up and the following items cultured: (i) Two large bore needles. (ii) One fine needle. (iii) Two lengths of plastic tubing. (iv) One syringe adaptor.

Packing and testing of samples

Infected threads were put into new latex gloves taken from a single batch from one manufacturer, and the gloves heat-sealed into bags of thin polythene or nylon sheet. Similar packaging was used for the samples of plastic tubing.

As radiation damage is associated with oxidation processes, it was to be expected that these effects could be reduced by irradiation *in vacuo*. In order to investigate a technique suitable for routine use, some of the gloves were vacuum-packed in plastic bags in the normal type of equipment used for food packaging.*

The average elongation and stress at breaking point for samples of rubber from irradiated gloves were determined for us by Dr R. H. Müller (Franklin and Sons Ltd., Dalston, London, E. 8). No quantitative measurements were made with the plastic tubings, as it was known that no significant radiation damage was produced at the dose levels used (e.g. Bopp & Sisman, 1955).

Irradiation

The majority of the test objects were irradiated for us by arrangement with Dr D. Powell at the Atomic Energy Authority Radiation Laboratory at Grove, Wantage, using their fuel rod gamma radiation source. Some of the gloves were exposed to electrons from a 2 MeV. Van de Graaff Unit by arrangement with Dr Black of Callenders Cables Ltd., Shepherds Bush, London, W. 12. The doses used were from 0·5 to 30 Mrad.

RESULTS

Bacteriological results

The use of 20 or more threads for each dose makes possible a type of 'dilution count' of the surviving organisms, for although no quantitative information is provided if 100 % of the threads remain fertile, lower percentages of fertile threads provide an estimate of the mean number of viable organisms per thread: 90 % fertile ≡ 2·3 organisms per thread; 63 % ≡ 1·0 organism per thread; 40 % ≡ 0·5 organism per thread.

The results in Table 1 show that in no test were 100 % of threads fertile after a dose of 1·5 Mrad., indicating that this treatment had reduced the viable count by at least 10,000-fold and that another 1·0 Mrad. (i.e. a total of 2·5 Mrad.) might be expected to provide a further approximately 500-fold reduction.

No significant differences were observed between the sterilizing effects of electrons and gamma rays or between sterilization in air and in the comparatively low vacuum obtained in the plastic bags. Neither the steel of a hypodermic needle nor the brass of its mount afforded protection for spores in its lumen under gamma irradiation.

The test threads were heavily contaminated, but the tube-and-needle assembly carried such contamination as might occur in used, but unwashed surgical apparatus; these objects were all sterilized by 0·75 Mrad. We may conclude that a dose of 2·5 Mrad. applied to carefully cleansed, and therefore lightly contaminated, objects would provide a large margin of safety.

Radiation damage

Gloves in the first batch were of the thin type with roughened fingers and palms, which are favoured by our surgeons. These were cold-vulcanized rubber solution-

* This packaging was kindly arranged by Mr J. M. Davies of T. Wall and Sons Ltd., Isleworth, Middlesex.

Table 1. *The results of culturing test objects after exposure to radiation*

				Dose (Mrad.)						
				0	0·5	0·75	1·0	1·5	2·0	2·5
Organisms	Approx. no. per thread	Radiation	Special conditions	Proportion of $\frac{1}{2}$ in. threads fertile						
B. globigii	10^5	Electrons	—	40/40	39/40	—	33/40	—	0/40	—
B. globigii	10^5	Gamma radiation	—	40/40	—	40/40	—	16/40	0/40	—
B. globigii	10^5	Gamma radiation	Vacuum packed	20/20	—	—	20/20	12/20	0/20	—
B. stearothermophilus	10^4	Gamma radiation	—	20/20	20/20	—	2/20	0/20	0/20	0/20
Cl. tetani	10^5	Gamma radiation	—	20/20	20/20	—	7/20	0/20	0/20	0/20
Cl. sporogenes	10^5	Gamma radiation	—	20/20	20/20	—	9/20	0/20	0/20	0/20
Cl. tetani	—	Gamma radiation	Long threads inside needles	2/2	2/2	—	1/2	0/2	0/2	0/2
Cl. tetani	—	Gamma radiation	Tubing and needles*	Growth	—	Sterile	Sterile	—	Sterile	—

* Infected by flushing through with blood containing spores of *Cl. tetani* 10^6/ml.; three needles, one metal adaptor, two lengths of polythene tube tested at each dose.

dipped gloves (B.S. 1803, type A). The results listed in Table 2 are the averages of five tensile tests on each glove, but with these rough gloves tests could only be made with material from the smooth cuff, whereas damage appeared to be noticeable first on the fingers where the glove was creased. Apart from one anomalous result for 10 Mrad., there was a progressive fall in breaking load and extension with increasing dose, the gloves showing significant reduction in strength after

Table 2. *Radiation damage after irradiation of rough surgical rubber gloves*

Dose (Mrad.)	Condition	Average stress at break (lb./sq.in.)	Average elongation at break (%)
British Standard	—	2000	600
No dose	—	2330	690
0·5	Slight smell	2670	725
1	Smell, darkening	2500	700
3	Smell, darkening	2300	700
4	Smell, darkening	1940	675
8	Smell, darkening	1100	650
10	Smell, darkening, punctured	2400	675
16	Smell, darkening, punctured	190	566
20	Smell, darkening, punctured	830	650
30	Badly perished	—	—

Samples were taken from smooth cuff of glove for tensile tests.

Table 3. *Radiation damage for smooth surgical rubber gloves*

Dose (Mrad.)	Average stress at break (lb./sq. in.)	Average elongation at break (%)
Control	3000–3400	750
4	2950	750
8	2700	725
10	2650	725
20	2350	675
30	2050	625

Samples taken from palm and fingers for tensile test.

only 8 Mrad. The radiation damage will always be related to the total accumulated dose, so that not more than three sterilizing doses of 2·5 Mrad. (as suggested above) would seem to be practicable.

However, the amount of radiation damage may be expected to vary considerably with the thickness of the glove and type of rubber and filler used. Thus, surgeons' gloves of the smooth type, but of the same nominal thickness and from the same manufacturer, proved to be stronger initially and, although there was a fall in breaking strength with increasing dose, the results were still above the British Standard specification after 30 Mrad. (Table 3). With this type of glove it was possible to include samples from the fingers in the tensile tests. These results emphasize that tests would be necessary to check the extent of damage with each type of glove used and there may even be some variation from batch to batch.

Results of similar tests made on gloves which had been vacuum-packed before irradiation are listed in Table 4 and show that the damage was markedly reduced by irradiation *in vacuo*. The rough gloves were still satisfactory after a dose of 16 Mrad. (corresponding to some six sterilization doses). This applied even when irradiation took place several months after packaging so that the vacuum was maintained satisfactorily at any rate for this length of time. The smooth gloves showed no appreciable loss of strength after 30 Mrad.

Table 4. *Radiation damage for smooth and rough surgical rubber gloves after vacuum-packaging*

Type of glove	Dose (Mrad.)	Average stress at break (lb./sq. in.)	Average elongation at break (%)
Rough	16	2160	625
Rough	16*	2060	650
Rough	4×4	1830	616
Smooth	10	3400	700
Smooth	20	3300	675
Smooth	30	3150	650

* Vacuum-packed 3 months prior to irradiation.

To simulate the practical conditions of repeated sterilization, some gloves were given four successive doses of 4 Mrad. in a vacuum pack, the gloves being worn, washed and dried in between each exposure. These results are included in Table 4, and it appears that the damage observed was slightly greater than for a similar total dose given as a single exposure. However, both the smooth and rough gloves can be considered as usable after 16 Mrad. delivered in four fractions. Some six repeated sterilizations with 2·5 Mrad. would, therefore, be practicable when using vacuum-packed gloves.

After exposure to 2 Mrad., vinyl tubing was found to show a definite yellow coloration which increased with dose. Nylon tubing showed no such colour change, and with neither type of tubing was there any apparent damage or alteration in properties, at any rate up to doses of 20 Mrad. Plastic tubing used for spinal anaesthesia, intravenous injection in infants, blood transfusion, or administration of radioactive isotope solutions would, in general, be disposable so that the problem of resterilization would not arise. With these materials, however, radiation damage does not present any problems.

Radiation indicators

The colour produced in vinyl plastic material has, in fact, been used for measurement of doses in the range 0·5–6 Mrad. (Artandi & Stonehill, 1958). We feel that it is desirable to be able to include in the sealed package some indicator to confirm that it has been irradiated, to at any rate the correct order of dose for sterilization. Tests have been made with thin polyvinyl chloride sheet as suggested by Artandi & Stonehill. We have used sheet 20 or 30 thousandths of an inch thick provided by

Bakelite Ltd., Birmingham (Types VR. 202 and DVR. 256) and these appear to be satisfactory for this purpose. The sheet is normally quite clear. After 1 Mrad. there is a slight, hardly detectable yellow colour, but this colour is very noticeable after 2 Mrad. The colour density increases with further dose, but an obvious yellow coloration of a small square of such material included in the sealed transparent plastic package would serve to indicate that a dose of at least 2 Mrad. (i.e. the order of the sterilization dose) had been received. Other plastic materials with suitable indicators could, of course, be used in a similar manner.

DISCUSSION

A large firm preparing suture material has, after extensive tests, adopted 2·5 Mrad. as a safe sterilizing dose (Artandi & van Winkle, 1959), and the present work confirms the adequacy of this dose.

From our results it appears that radiation sterilization of rubber gloves and plastic tubing is practicable, but, at any rate, with some types of rubber glove, the severe radiation damage makes repeated sterilization impossible. This would not be an objection if the gloves could be produced cheaply as a disposable product, prepacked and sterilized by the manufacturer. Further, the use of disposable gloves would avoid the cost of labour expended on cleaning, testing, repairing, dusting and packing gloves in the operating theatre. However, theatre staff, who are on duty but not occupied with actual surgical operations, are often available for this work. Also, surgeons' gloves are at present relatively expensive and each pair is probably used, on the average, about five times. It would appear, therefore, that the price will have to be considerably reduced for a disposable glove if the cost is not to be appreciably increased.

If it is required to carry out repeated sterilization on the rubber gloves at present available, the radiation damage can be reduced by vacuum-packaging before irradiation. Alternatively, it is possible for manufacturers to produce a glove incorporating chemical protective agents ('antirad') which would effectively reduce the radiation damage. In the former case it is an added advantage that the preservation of the vacuum (the packages remaining stiff) provides a check of the integrity of the bag, although it is, of course, possible for the vacuum to be lost, through a gas leakage, without the contents having had an opportunity to become infected.

Radiation sterilization is not, in general, suitable for repeated application to materials such as wool or cotton or rubber sheeting with a textile base, as in these the radiation damage is considerable (Ellis, Oliver & Vollum, 1959). Instruments, glass syringes, etc., can be sterilized satisfactorily by heat. Thus, the special application of radiation sterilization in the hospital would be for rubber gloves or plastic tubing as discussed above and for other plastic materials such as blood-transfusion equipment and Petri dishes. For such limited use it is unlikely that a radiation unit could be economically utilized except in a central sterilization organization covering a large hospital or a group of smaller hospitals. In some special instances where a high energy electron accelerator is available (for instance, for Radiotherapy) the part-time use of such a source for sterilization could be considered.

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The alternative application of radiation sterilization is in the supply by manufacturers of presterilized packages of such disposable items as gloves, catheters, plastic tubing, blood transfusion sets and dressings.

The use of ionizing radiation can provide a simple and reliable means of sterilization inside completely sealed packages (thus reducing the possibility of re-infection during storage), and its practical use should be further investigated by the installation of pilot equipment in a few hospitals.

SUMMARY

Threads were infected with the spores of four species of bacteria and put inside rubber gloves which were sealed into plastic bags and irradiated with electrons or gamma rays. A dose of 1·5 Mrad. killed approximately 99·99 % of the spores of each species and a dose of 2·5 Mrad. appeared to give an adequate margin of safety for sterilization. Spores were similarly killed inside plastic tubing and within the lumen of hypodermic needles.

The tensile strength of the gloves decreased with increasing doses of radiation so that the rough, solution-dipped gloves tested were significantly weaker after 8·0 Mrad., but the smooth gloves tested still complied with the British Standard after 30 Mrad. When vacuum-packed before irradiation, rough gloves were still satisfactory after 16 Mrad., and smooth gloves were apparently unaffected by 30 Mrad.; they could, therefore, be sterilized six and twelve times, respectively.

Radiation gives dependable sterilization of rubber gloves, and the use of a sealed plastic package obviates subsequent contamination. The possibility of using disposable radiation-sterilized gloves is discussed, the use of a plastic radiation indicator suggested and the practical applications of radiation sterilization in the hospital considered.

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REFERENCES

Artandi, C. & Stonehill, A. A. (1958). *Nucleonics*, **16**, no. 5, 118.
Artandi, C. & van Winkle, W. (1959). *Nucleonics*, **17**, no. 3, 86.
Bopp, C. D. & Sisman, O. (1955). *Nucleonics*, **13**, no. 10, 51.
Bowie, J. H. (1957). *Hosp. Engr.* **11**, 98.
Darmady, E. M., Hughes, K. E. A. & Jones, J. D. (1958). *Lancet*, ii, 766.
Edwards, R. B., Peterson, L. J. & Cummings, D. G. (1954). *Food Tech., Champaign*, **8**, 284.
Ellis, F., Oliver, R. & Vollum, R. L. (1959). *Brit. J. Radiol.* **32**, 280.
Foster, F. L., Dewey, D. R. & Gale, A. J. (1953). *Nucleonics*, **11**, no. 10, 14.
Fuld, G. J., Proctor, B. E. & Goldblith, S. A. (1957). *Int. J. Appl. Radn. & Isotopes*, **2**, 35.
Miller, C. W. (1955). *Metropolitan Vickers Research Series* no. 12.
Pepper, R. E., Buffa, N. T. & Chandler, V. L. (1956). *Applied Microbiol.* **4**, 149.
Report of the Central Pathology Committee on the Sterilization of Hospital Equipment (1954). Ministry of Health.