The survival of *Streptococcus pyogenes* on bacteriological swabs made from various fibres

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The failure of bacteria to survive on cotton-wool swabs has been attributed to the presence of toxic materials in the wood sticks (Beakley, 1957), the low pH of processed cotton-wool (Anderson, 1965), the presence of fatty acids (Pollock, 1947, 1948; Rowatt, 1957) and, when the swabs have been irradiated, to the formation of toxic products during this process (Mair and McSwiggan, 1965; White, 1965). To increase the time of survival, Rubbo and Benjamin (1951) coated the swabs with serum, and Stuart, Toshach and Patsula (1954) boiled them in phosphate buffer and then treated them with charcoal. Stuart (1946) devised a non-nutritive transport medium into which the cotton-wool swab was placed after sampling. Survival of a number of organisms was increased by the use of this technique.

Preliminary experiments showed that all samples of cotton-wool available for testing had a pH of about 6.0. However, the survival of *Streptococcus pyogenes* on swabs made from different samples varied enormously. This was not surprising, because cotton-wool is a natural product processed by cleaning, bleaching and treating in various ways and is manufactured by blending more than one type of cotton. The use of man-made or synthetic fibre might therefore provide a means of obtaining a more consistent product on which survival could be determined. Some materials of this type have been tried as a substitute for cotton-wool, including calcium alginate (Cain and Steele, 1953), Dacron (Hollinger and Rantz, 1959) and PVA cotton, Fortrel and Dacron (Ellner and Ellner, 1966). However, the results obtained were far from conclusive and the variability observed may be attributed to the different techniques used in the test procedure.

The object of this investigation was to determine the survival of streptococci on a number of commonly available fibres of a suitable nature for use as swab heads, to evaluate the effect of sterilisation by irradiation on survival and, finally, to examine the factors likely to affect the results obtained in tests of swabs for the presence of toxicity towards bacteria.

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MATERIALS AND METHODS

Test procedure

This was based on the method of Rubbo and Benjamin and the full details are as follows.

Organisms. Four strains of group-A streptococci were used. Three (strains A, B and C) were isolated at the Public Health Laboratory at Shrewsbury and the fourth (strain D) was obtained from the laboratory collection at Imperial College.

Preparation of inoculum. Suspensions for the inoculation of the swabs were prepared in one of two ways designated as "agar-grown" and "broth-grown" in the text.

Agar-grown streptococci were prepared by inoculating the organisms on to slopes of blood agar (Oxoid Nutrient Agar with 7 per cent. horse blood) and incubating for 18 hr at 37°C. The organisms were then removed from the agar surface with an inoculating loop and transferred to a bottle containing 0.1M phosphate buffer, pH 7-0. The suspension was shaken vigorously; microscopical examination showed that after this the majority of organisms existed as single cells, with the remainder in pairs. Finally the suspension was adjusted by the addition of phosphate buffer to give plate counts of about 1 x 10^7 colonies per ml.

Broth-grown streptococci were obtained by growing the organisms in Hartley's digest broth containing 0.2 per cent. dextrose. After incubation overnight at 37°C, the cells were harvested by centrifugation and the organisms resuspended in a similar volume of fresh sterile Lemco broth without dextrose. Three sterile glass beads were added and the suspension was shaken by hand for 30 s. It was then diluted with fresh sterile Lemco broth to give plate counts of about 1 x 10^5 colonies per ml.

Inoculation of swabs. Each swab was inoculated individually (after irradiation for those treated in this way) by delivering either 0.1 ml of the suspension from a 0.2 ml pipette (for agar-grown inocula) or 0.02 ml from a dropping pipette (for broth-grown inocula) on the swab head. Initially, the inoculum was applied in one spot on the swab head, but with some fibres of low absorbency it was difficult to get 0.1 ml taken up and some distribution of the inoculum over the swab head was necessary. Subsequently, it was found that differences in the survival of the test organism depended on whether the inoculum was distributed over the whole head or delivered in one spot. Therefore, in the experiments recorded in this paper, where the volume of the inoculum was 0.1 ml it was applied to the swab so that it was evenly distributed over the whole head, by slowly delivering the required volume from the pipette while the swab was rotated. Where an inoculum of 0.02 ml was used, all that was possible was to apply it as a single drop in one spot.

Storage of inoculated swabs. Swabs that were not to be sampled at once were stored singly in 150 x 16 mm Pyrex glass test-tubes, the swab being held in place by a cotton-wool plug. Care was taken to see that the swab head did not touch the side of the tube. The tubes were kept at laboratory temperature away from direct sunlight.

Sampling. Counts were made of the number of colony-forming units of streptococci recoverable from swabs immediately after inoculation and 4 and 24 hr later. Each swab head was placed in 10 ml of 0.1M phosphate buffer, pH 7-0, or Oxoid Lemco broth contained in a 25-ml McCartney bottle, the end of the stick was broken or cut off and the bottle was then shaken vigorously for 30 s to break up the head and distribute the fibres. Ten-fold dilutions in phosphate buffer or Lemco broth were made from this and the number of surviving streptococci was determined immediately by the surface viable count method; nine drops of 0.02 ml were plated on to the surface of each of two dry blood agar plates for each dilution. The colonies were then counted after incubation for 18-20 hr at 37°C. The results were expressed as the percentage survival after 4 and 24 hr. Each figure given in the Results section is the mean of at least 20 replicate counts.

Preparation of swabs

Materials. The origin, type and nature of the fibres used for the preparation of swabs are given in the table. This also indicates whether the swabs were factory-made or made in the laboratory. Materials with the same trade description but obtained from different suppliers
are referred to as different "samples", e.g., factory-made rayon swabs of samples 1 and 2. Rayon swabs obtained from the same supplier on two different occasions are described as rayon (sample 1) "batches" X and Y.

Making the swabs. In the majority of cases, the swabs were made in the laboratory from bulk "wool" and paper, wood or stainless-steel sticks. Wood sticks were preferred for ease of sampling, but paper sticks were frequently used as they were readily available. Preliminary tests showed that the survival on swabs prepared from any one fibre was not affected by the composition of the stick. A small piece of the "wool" was wound on to the end of the stick by rotation. No adhesive was used, and with little effort swabs very similar to those made commercially could be prepared. The swabs were sterilised by heat or irradiation only where this is specifically stated in the text.

<table>
<thead>
<tr>
<th>Swab made in</th>
<th>Type of material</th>
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<tr>
<td>Factory*</td>
<td>Synthetic fibre</td>
</tr>
<tr>
<td>Laboratory</td>
<td>Ulstron (polypropylene)†</td>
</tr>
<tr>
<td></td>
<td>Terylene†</td>
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<td></td>
<td>Nylon‡</td>
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<td></td>
<td>Dacron‡</td>
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* Obtained from four different manufacturers; † supplied by Imperial Chemical Industries Ltd; ‡ supplied by DuPont Ltd; § supplied by Courtaulds Ltd; ¶ supplied by Medical Alginates Ltd; ‡ supplied by Johnson and Johnson Ltd; ** obtained from Southalls (Birmingham) Ltd; †† available in the laboratory and labelled "Zorbo" superfine absorbent cotton-wool.

Irradiation. The swabs were packed in paper or polythene bags, or alternatively in Pyrex glass tubes, wrapped in brown paper before being irradiated. This was carried out by exposure to a cobalt-60 source or by passage through a linear accelerator. Except when specifically varied in certain experiments, the dose given was 2.5 MR.

RESULTS

Survival on unirradiated fibres

The survival of strain D (agar-grown streptococci) on unsterilised swabs made from 11 of the 14 fibres tested is shown in fig. 1. The materials may be classified into two groups, those on which the viability remained high after 24 hr, which included rayon (sample 1), Ulstron (polypropylene), cotton-wool (sample 3), Dacron, surgical absorbent (rayon), viscose rayon, Fibro SW and Triciel, and those on which it dropped to a low level, which included calcium alginate, cotton-wool (samples 1 and 2), rayon (sample 2), Nylon and Terylene. Calcium alginate and cotton-wool (sample 1) were notably poor and viability was substantially reduced even after only 4 hr.
FIG. 1.—Percentage survival of *Str. pyogenes* strain D (agar-grown inoculum) on swabs made of various unsterilised fibres.
Survival on pre-irradiated fibres

The survival of agar-grown streptococci of strain D on six of the fibres before and after pre-irradiation by means of a cobalt-60 source with a dose of 2-5 MR (that generally recommended for sterilisation) is shown in fig. 2. Viability on each of the irradiated fibres except Ulstron was lower than on the corresponding unirradiated fibre. The effect on rayon (sample 1) was particularly pronounced, leading to a reduction from 82 per cent. viability for the unirradiated fibre to 1 per cent. for the irradiated fibre at 24 hr. However, as will be shown later, not all batches of rayon (sample 1) exhibited this pronounced effect, although in all cases irradiation resulted in some reduction in viability. In the case of Terylene, cotton-wool (sample 1) and calcium alginate, the low viability recorded for the unirradiated fibre was further reduced by irradiation, making them the least suitable of the fibres tested.

The effect of irradiation dose was determined by subjecting swabs prepared from rayon (sample 1) to 1.25, 2.5 and 5.0 MR from a cobalt-60 source. The survival of strain D on these indicates that over the range tested loss of viability of the test organism was dependent on the dose of irradiation given. This is consistent with the assumption that toxic products arise during irradiation and that their accumulation is responsible for the loss of viability.

As an alternative method, swabs prepared from various fibres were irradiated with a dose of 2.5 MR by means of a linear accelerator. The results indicated that swabs treated in this way were less toxic for streptococci than those irradiated from a cobalt-60 source, but the number of tests done was insufficient for an assessment of the size of the difference to be made.

Effect of irradiation on different batches of rayon

Though fibres wholly synthesised by the organic chemist can be made extremely consistent in composition and purity, this may not be true of man-made fibres such as rayon that are prepared from a regenerated natural product. During the course of this investigation, two distinct batches of rayon (sample 1) swabs were examined. Both were supplied as identical products, the only difference being that one was obtained approximately 8 mth after the first.

The viability of agar-grown streptococci of strain D on the two batches (X and Y), irradiated and unirradiated, is shown in figs. 3 and 4. Before irradiation the batches behaved similarly, but afterwards batch X was very much inferior to batch Y, which had little inhibitory activity even in 24 hr. The reason for this is not clear, but it might be due to the intermittent presence of a contaminating material in the cellulose that is converted into a more toxic product during irradiation.

Susceptibility of different strains of streptococci

The survival of agar-grown cells of three strains of streptococci on irradiated rayon (sample 1, batch Y) was compared (fig. 5). Strain D, which had been used in all the previous studies, proved to be much more resistant than
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FIG. 2.—Percentage survival of Str. pyogenes strain D (agar-grown inoculum) on unirradiated and irradiated swabs made from six different fibres.
strains A and B to the toxic products in cobalt-60-sterilised material. The most sensitive streptococcus (strain A) showed more variation between replicate swabs in a single experiment and between replicate experiments than did strain B, and for this reason is not to be regarded as suitable for use in routine tests.
Similar results were obtained on irradiated Terylene, irradiated cotton-wool (sample 1) and irradiated calcium alginate, although the differences in sensitivity between different strains varied in amount with the material tested.

Sterilisation of cotton-wool swabs by heat

It has been suggested that cotton-wool with toxic properties may be rendered non-toxic by autoclaving, and that the same fibre becomes more toxic after dry-heat sterilisation. This was investigated with cotton-wool (sample 1) which was relatively inhibitory in the unsterilised state. The survival of broth-grown streptococci of strain C on untreated swabs and on swabs that had been autoclaved at 137°C for 3½ min. or hot-air-sterilised at 160°C for 1 hr, is shown in fig. 6. Neither method of sterilisation influenced survival after 24 hr, though survival at 4 hr was somewhat reduced by hot-air sterilisation. There was no evidence of the reduction in the toxicity of the cotton-wool by autoclaving.

Pre-treatment of cotton-wool swabs with buffer or serum

Swabs made from cotton-wool (sample 1) were treated with phosphate buffer, pH 7.0 in one of two ways. In the first, the swabs were dipped in the buffer and then dried at 75°C for 1 hr, and in the second, they were boiled in the buffer for 5 min. before being dried in the same way and then autoclaved. The survival of broth-grown streptococci of strain B on these swabs was determined and was compared with the untreated autoclaved control. The results (fig. 7) show that though both treatments substantially increased the survival of the test organism, boiling was more effective than soaking in buffer and resulted in nearly 15 per cent. survival after 24 hr.

The factory-made swabs, cotton-wool (sample 2), are not sold in their raw state, but are marketed either after treatment with phosphate buffer or after coating with serum. The effect of these treatments on the survival of broth-grown cells of strain B was investigated. Survival after 24 hr on untreated swabs was less than 0.01 per cent.; on serum-coated swabs it was 14.8 per cent. and on plain swabs that had been boiled in phosphate buffer it was 14.3 per cent. It appeared therefore that the two treatments are equally suitable as methods of increasing the survival of streptococci on cotton-wool swabs.

Survival of streptococci after the dissolution of calcium alginate swabs

It is clear that calcium alginate is an unsuitable fibre for use as a dry swab, but the possibility was considered that immersion of the swab immediately after inoculation in the fluid used to dissolve this fibre might increase the survival of streptococci. The survival of agar-grown streptococci of strains B and D on cobalt-60-irradiated calcium alginate swabs immersed in a solution containing 0.5 per cent. (w/v) sodium hexametaphosphate and 0.5 per cent. (w/v) sodium citrate and shaken vigorously, was therefore determined after storage for 4 and 24 hr. The results are shown in fig. 8. Strain D survived well under these conditions; the percentage survival at 24 hr was 17.7 after solution of the fibre and 0.022 on the comparable untreated material (see fig. 2). On the other
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hand, a more sensitive streptococcus (strain B) died out more rapidly after solution of the fibre (percentage survival 0.23), but its survival on dry irradiated alginate swabs was even less (under 0.01 per cent.). It appeared, therefore, that solution of the swab did result in some increase in survival, but that this was not sufficient to give acceptable recovery of the more sensitive streptococci.

Moistening of swabs with phosphate buffer after inoculation

As an alternative to the use of a transport medium, the effect of moistening the swab with phosphate buffer was examined. The survival of agar-grown streptococci of strain D on Terylene swabs, irradiated and unirradiated, and with and without the addition of phosphate buffer to moisten them immediately after inoculation, was determined. Only just sufficient buffer (c. 0.1 ml) was delivered from a capillary pipette to moisten the swab so that moisture could be observed in the outer layer of fibres. Fig. 9 shows that there was a pronounced increase in survival on both irradiated and unirradiated swabs when they were moistened with buffer. Survival on irradiated moistened swabs was nearly 30 per cent. after 24 hr, compared with less than 0.01 per cent. for the irradiated control. Similar results were obtained with rayon (sample 1), although the effect of the buffer was less pronounced because of the higher survival of streptococci on this fibre.

Survival of agar-grown and broth-grown streptococci

In the present investigation two different techniques were used to prepare bacterial inocula. In order to find out whether the method of preparation of

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**Fig. 7.**—Percentage survival of *Str. pyogenes* strain B (broth-grown inoculum) on swabs of cotton-wool (sample 1) that had been boiled or soaked in phosphate buffer (pH 7.0) before being sterilised by autoclaving. ○—O Un-treated; □—□ soaked in buffer; Δ—Δ boiled in buffer.

**Fig. 8.**—Percentage survival of two strains of *Str. pyogenes* (agar-grown) inoculated on to irradiated calcium alginate swabs that were subsequently dissolved in 0.5 per cent. sodium hexametaphosphate and 0.5 per cent. sodium citrate. ○—○ Strain D; Δ—Δ strain B.
the inoculum influenced the subsequent survival of streptococci on the swabs, agar-grown and broth-grown inocula of strain B were placed on swabs of irradiated rayon (sample 1). The percentage survival at 4 hr and 24 hr is shown in fig. 10.

Although there was a very substantial difference in the survival after 4 hr, the difference after 24 hr was relatively small. It appeared that there was a more rapid loss of viability in the first 4 hr when agar-grown streptococci were suspended in phosphate buffer than when broth-grown streptococci were suspended in broth, but that, after the initial loss of viability, survival was slightly better in the former than in the latter case. The reason for this is not certain, but it may in part have been a consequence of using a colony-counting method to measure the number of surviving organisms. The chain length of the streptococci was greater in the broth-grown than in the agar-grown inocula, so that more of the former than of the latter would have to be destroyed before the colony count was reduced (see Lidwell, Noble and Dolphin, 1959).

**DISCUSSION**

Although cotton-wool has been used in the manufacture of swabs for a long time, its suitability for this purpose has only recently been considered critically. The poor survival of bacteria on cotton-wool swabs has frequently been reported, but a comparison of the results obtained by different workers is not possible in the absence of a standardised testing procedure. The method of Rubbo and Benjamin (1951) appears to be broadly satisfactory for use with *Str. pyogenes*, but we have found that a number of factors, including the strain

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**Fig. 9.**—Percentage survival of *Str. pyogenes* strain D (agar-grown inoculum) on unirradiated and irradiated Terylene swabs that had been moistened with phosphate buffer (pH 7-0) after inoculation. Unirradiated, untreated; △- -△ irradiated, untreated; ○- -○ unirradiated, moistened with buffer; •--• irradiated, moistened with buffer.

**Fig. 10.**—Survival of agar-grown and broth-grown inocula of *Str. pyogenes* strain B on irradiated swabs of rayon (sample 1). ○- -○ Broth-grown; △- -△ agar-grown.
of the test organism, the distribution of the inoculum on the swab head and the technique used in the preparation of the inoculum, affect the results obtained by this technique. The two methods of preparing the inoculum described in this paper both gave reproducible results with all the fibres tested, but the use of agar-grown streptococci resulted in a more sensitive test for bactericidal activity during the first 4 hr of contact with the fibre.

A suitable test organism is one that is relatively sensitive to the inhibitory substances present in swabs and also gives reproducible results in replicate tests. Although very sensitive strains, such as strain A, could be easily found, they always exhibited poor reproducibility when tested for survival on any fibre. Variation between replicates was frequently ten times greater than that observed with less sensitive strains such as strain B, which themselves were shown to be much more sensitive than strain D. Strain B appears to be a suitable test strain, combining good sensitivity with the ability to give reproducible results.

Survival of streptococci in inhibitory batches of cotton-wool swabs could be improved by boiling the swabs in phosphate buffer or dipping them in serum before use, or by saturation of the swabs with phosphate buffer after sampling. In the latter case the swabs remained moist for 24 hr, but the increased survival of the streptococci must be attributed to the presence of the phosphate buffer rather than to the prevention of drying, because when similar swabs without added phosphate buffer were stored in a saturated atmosphere they also remained moist for the whole 24 hr, but showed little increase in survival. It appears that slow drying is not the major cause of loss of viability under the conditions used in our experiments.

Hosty et al. (1964) showed that the survival on Dacron and cotton swabs of Str. pyogenes in throat samples was increased if the swabs were dried rapidly over silica gel and Redys, Hibbard and Borman (1968) further demonstrated this effect on cotton swabs when the organisms were suspended in rabbit serum and stored at a temperature of 86°F (30°C). Apparently at 50°F (10°C) slow drying had little effect as all samples remained viable for up to 72 hr.

On all the unirradiated fibres tested, with the exception of cotton-wool (sample 1) and calcium alginate, the survival of Str. pyogenes exceeded 10 per cent. after 4 hr. Swabs made with these fibres would probably have given acceptable results when the time between swabbing and culturing did not exceed 4 hr. But some doubt must exist about the length of time that can safely be allowed to elapse in practice. Five out of the six fibres tested were more inhibitory for Str. pyogenes after irradiation. The effect of irradiation varied in magnitude from fibre to fibre and, in the case of rayon, from batch to batch. In every case survival on the irradiated fibre was low after 24 hr when a sensitive streptococcus was used as the test organism.

The clinical microbiologist can therefore be confident that he will obtain satisfactory results only if he carries out regular batch-testing on the fibres used for the manufacture of swabs, or if he obtains evidence that such tests have been made. When swabs with inhibitory properties have to be used, pretreatment with serum or boiling in phosphate buffer may make them acceptable.
Alternatively, a transport medium may be used. Saturation with phosphate buffer after sampling, though effective in our hands, cannot be regarded as a practical alternative.

Calcium alginate fibre falls into a special category because of its solubility in certain solutions. The advantages claimed for this fibre are that, since it is soluble in body fluids, any fibres that become detached when the sample is taken will be dissolved away, and that all the organisms on the swab can be recovered by immersing it in solvent. We have shown that the viability of \textit{Str. pyogenes} is rapidly lost on this fibre and immersion in a solvent medium immediately after inoculation is not effective in reducing this loss of viability substantially.

**Summary**

The survival of \textit{Streptococcus pyogenes} on swabs varies with the fibre used. In the case of rayon sterilised by irradiation, batch-to-batch variation also occurs, and this indicates that batch testing of fibres is essential. Survival is reduced on swabs that have been sterilised by gamma irradiation, but is relatively unaffected by heat sterilisation. With cotton-wool swabs, survival is increased by boiling the swab in phosphate buffer, by pre-treatment with serum or by saturation with phosphate buffer after sampling. The strain of the test streptococcus, the mode of application of the inoculum and the method used to prepare the inoculum all affect the experimental results.

**REFERENCES**