During the first six weeks each group of three pigs received alternately the saline extracts of "splenin A" and of "splenin B" from pooled normal serum. During the last six weeks they received extracts of "splenin A" and of "splenin B" from pooled rheumatoid serum. The results are recorded in Table III and Table IV.

TABLE V.—Analysis of Variance (Experiment 2)

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio	Probability
Variation due to characteristic bleeding-times of each guinea-pig	1,403	5	281	0.30	N.S.
Variation due to characteristic day effects on the bleeding-time	7,805	10	781	0.85	N.S.
Variation among each set of four observations taken in sequence	401,712	432	936	1.01	N.S.
Effects of Treatments:					
Variation among the four groups of guinea-pigs before treatment	3,267	3	1,089	1.18	N.S.
Decrease in bleeding-time due to:					
Normal serum extract A	13,806	1	13,806	14.96	0.001 > P
Normal serum extract B	1,008	1	1,008	1.09	N.S.
Rheumatoid serum extract A	160	1	160	0.17	N.S.
Increase in bleeding-time due to rheumatoid serum extract B	17,600	1	17,600	19.07	0.001 > P
Residue remaining after removal of the above effects regarded as error arising randomly	111,731	121	923	—	
Total variation from all sources	558,492	575			

The data in Tables III and IV gave the variance analysis shown in Table V. From this it can be seen that the six guinea-pigs were more uniform than those used in the first experiment (variance ratio=0.85, $n_1=5$, $n_2=121$, not significant). The twelve occasions on which the experiment was carried out did not give rise to differences in bleeding-times (variance ratio=0.85, $n_1=10$, $n_2=121$, not significant). The four groups of observations before treatment did not vary more than would be expected ($F=1.18$, $n_1=3$, $n_2=121$, not significant). After treatment with serum extracts the groups differed. Table VI shows the differences.

TABLE VI

Treatment	Initial Bleeding-time	Alteration in Bleeding-time	Responding Bleeding-time
Normal serum extract A	122 seconds	Decreased 20	102 seconds
Normal serum extract B	128 "	" 5	123 "
Rheumatoid serum extract A	122 "	" 2	120 "
Rheumatoid serum extract B	129 "	Increased 22	151 "

and from the variance analysis (Table V) it appears that normal serum extract A decreases the bleeding-time (variance ratio=14.96, $n_1=1$, $n_2=121$, $P=0.001$) and rheumatoid serum extract B increases it (variance ratio=19.07, $n_1=1$, $n_2=121$, $P=0.001$). The remaining two treatments were ineffective at the dose given (variance ratio=1.09 and 0.17, respectively, $n_1=1$, $n_2=121$, not significant).

Conclusions

It is concluded that a factor or group of factors present in the "splenin A" extract of normal serum reduces bleeding-time, but is absent from a similar

extract from rheumatoid patients. A factor or group of factors is present in the "splenin B" extract of rheumatoid serum that increases bleeding-time, but is absent from normal serum. Both effects have been found consistently.

The experiments, while confirming the existence of the substances called by Ungar "splenin A" and "splenin B," give no indication of the manner or place of production of these substances. His view that they are produced in the spleen and that their production forms part of the general adaptation syndrome is at present under investigation.

We wish to express our thanks to Dr. G. Ungar for initiating us into his methods of extracting the "splenins"; to Mr. A. L. Bacharach and Mr. B. Basil, both of Glaxo Laboratories Ltd., for their help in planning this experiment and undertaking the statistical analysis, respectively; and to Dr. W. J. Martin, of the Statistical Research Unit, London School of Hygiene and Tropical Medicine, for his helpful criticism.

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THE STERILIZATION OF WOOLLEN FABRICS

BY

H. F. BARNARD, M.B., B.S.

Pathologist, Westwood Hospital, Beverley, E. Yorks.

Air-borne infection is frequent in the case both of wounds and of infections of the respiratory tract. The infectious organisms are carried on dust or on droplets and droplet nuclei arising from the mouth and nose. Of the two, dust is probably responsible for greater degrees of air contamination (Hare and MacKenzie, 1946; Duguid and Wallace, 1948). Such infected dust arises for the most part from the movement of fabrics—for example, blankets, clothes (van den Ende, Lush, and Edward, 1940; Green, Challinor, and Duguid, 1945; Duguid and Wallace, 1948), bandages (Bourdillon and Colebrook, 1946), and handkerchiefs (Dumbell, Lovelock, and Lowbury, 1948), and to a somewhat less extent from floor-sweeping (Green, Challinor, and Duguid, 1945), although much of this floor dust has come in the first place from fabrics.

Dust-borne bacteria are relatively resistant to the bactericidal action of ultra-violet light and chemical aerosols (Twort, Baker, Finn, and Powell, 1940; Dumbell, Lovelock, and Lowbury, 1948). Correct ventilation can clear the air fairly rapidly (Bourdillon and Colebrook, 1946). To a large extent, however, control has depended upon preventing the release of dust, by oiling in the case of blankets (Thomas and van den Ende, 1941) and floors (van den Ende, Lush, and Edward, 1940), by adequate gowning in the case of clothes (Duguid and Wallace, 1948), and also, of course, by washing.

Relatively little attention has been paid until recently to the adequate bacterial cleansing of the fabrics whence comes so much of the pathogen-bearing dust, though the problem has been appreciated by workers in this field. Bourdillon and Colebrook (1946) state that, "whilst a freshly laundered gown or clean sheet is almost sterile, all woollen or flannel articles must be regarded as infected." Colebrook, Gibson, Todd, Clark, Brown, and Anderson (1944) reported finding haemolytic streptococci in freshly laundered blankets in a hospital and, at the same time, in burns, both organisms being of the same type. A similar experience led here to a convenient way of sterilizing blankets.

Cross-infection and Woollens

For some years efforts have been made to reduce the minor infection of clean wounds and similar cross-infections. Mostly these have been caused by the *Staphylococcus aureus*, the majority of strains in hospital cases being penicillin-resistant. Periodical checking of the staff has always shown that some 50% are nasal carriers of this organism, many of these, too, being resistant strains. However, phage-typing of the organisms recovered from patients and staff, kindly carried out by Dr. V. D. Allison, cast doubt on the direct nurse-to-patient route of spread, as the type in the wound often did not correspond with that found in the nurses in contact. Therefore, when the majority of freshly laundered blankets were found to contain *Staph. aureus*, it became desirable to find a convenient way of sterilizing these and closing this possible route of spread.

Woollens, as we know, shrink in hot water, so that though other fabrics are washed at a temperature lethal to most organisms, woollens are not so treated. As Bourdillon and Colebrook (1946) state: "Whilst clean linen and cotton goods are almost sterile, flannel and woollen goods are in a very different category, since in most laundries they are not heated to a bactericidal temperature." In the present case blankets are churned for 10 minutes at 43° C., and staphylococci in test squares of blanket survive the procedure readily. If a piece of blanket is heat-sterilized and then washed in the machine with the routine ward blankets and thereafter cultured, it is found to have picked up, in what appears to be full measure, the bacterial flora from the other blankets. Evidently transfer of organisms between blankets in the wash readily occurs. It is significant, therefore, that one has found occasionally used blankets to be grossly infected with staphylococci.

Sterilization of Blankets

The poor bactericidal results of laundering methods for woollens have led in recent times to attempts being made to find better ways. Bactericidal temperatures being detrimental to wool, attention has been turned to chemical disinfection.

Van den Ende and Spooner (1941) impregnated squares of fabric by soaking in various bactericidal solutions and, when dry, sprayed them with a culture of haemolytic streptococci or with an infected powder. Later the fabric was shaken over an agar plate or wiped on it. A number of bactericides were tried, but none was considered of practical value. Puck, Robertson, Wise, Loosli, and Lemon (1946) used a similar method, and though the fabric, when dried, retained much of the bactericide, the haemolytic streptococci sprayed on to it could later be recovered by culture. Contact between

the two was evidently insufficient, probably owing to the bactericide failing to spread readily over the fibres of the fabric. Rountree (1946) and Rountree and Armytage (1946) found that blankets destroyed bacteria when oiled by the method recommended by Harwood, Powney, and Edwards (1944). The bactericidal agent was the detergent cetyl pyridinium bromide (C.P.B.), which remained with the oil in the blanket. Haemolytic streptococci sprayed on to the treated fabric could not be recovered later by wiping it on agar or by washing it in broth. The fabric was not harmed in any way by the process, nor were a small group of people found to be sensitive to contact with the impregnated fabric.

Dumbell and Lovelock (1949) have studied disinfectants suitable for impregnating handkerchiefs to render them self-disinfecting. *Staph. aureus* in saliva was soaked into the treated cotton fabric, which, when dry, was shaken over agar plates. The most active agent tried, hexyl resorcinol, was of prohibitive cost, but octyl cresol and "alkyl" resorcinol were considered practicable. They considered that for handkerchiefs C.P.B. was unsuitable, as it had been reported by Williams, Clayton-Cooper, Duncan, and Miles (1943) that some people (under 1%) are sensitive to its local application.

Vaughan, Sotier, Hill, Simpson, and Lee (1948) examined ways to sterilize woollens in a laundry wash process. In this work there was no attempt at impregnation to render the fabric self-disinfecting. Chlorination was considered the most practical method, and the agent causing the least harm to dyes and fabric was sodium benzene sulphone chloramide ("monochloramine B"). This method was found suitable for the large-scale treatment of clothing in the American Army. Small fabric squares infected with *Bact. coli* and *B. subtilis* were included with the clothing treated, and were afterwards found to be sterile.

Before we became acquainted with the above relevant literature, consideration had been given to chemical disinfection of blankets. For reasons of cost, toxicity, damage to fabric, and so on, all but a few disinfectants appeared to be unsuitable. Trial use was made of calcium hypochlorite in the form of eusol powder over a period of some months, pending the discovery of a better way. It was, in the strength used (2.5% of eusol powder), less adequately bactericidal in the laundry than in the test-tube and, moreover, it produced slight but noticeable hardening of the wool.

C.P.B. seemed to deserve further consideration. It is a cation-active detergent, and as "fixanol C" is manufactured by I.C.I. The high bactericidal activity of certain of the surface active agents has been extensively examined since the publication of Domagk (1935). Quisno and Foter (1946) examined cetyl pyridinium chloride and found it unusual in being highly bactericidal over a wide range of pH. Van den Ende, Edward, and Lush (1941) had tried C.P.B. and found that, though it left the fabric bactericidal, it gave a "harsh texture and unpleasant odour." Rountree found fixanol in solution highly bactericidal, especially to Gram-positive organisms. She found that blankets soaked with oil and fixanol solution and dried were virtually sterile for many weeks: 1 in 800 in the blanket (in addition to the oil) was adequate, but 1 in 1,600 was much less so. Patch tests with 1% solution gave no reactions in 17 people.

The purpose of the oiling is to reduce dust release, and, while Rountree (1947) found that it brought no

reduction in surgical cross-infection, Wright, Cruickshank, and Gunn (1944) were able to show a reduction in cross-infection in measles wards. The process, however, is not without disadvantages. It is not very easy for a normal laundry to control, it is somewhat expensive, and the finished product is a slightly oily blanket—though the work of the U.S. Navy Medical Research Unit (1946) would seem to have overcome much of the trouble and cost. It seemed, however, that a trial of fixanol C. without oil would be worth making.

Present Investigation

The concentration necessary was suggested by the above oil and fixanol experiments—namely, one part by weight of undiluted fixanol to 800 parts by weight of blanket. It was found that, in the laundry after hydro-extracting, the water held in a blanket before air-drying averages one-third of its dry weight, a gain in weight of 33%. Thus, to attain a final 1 in 800 the blanket would have to be washed in a 1 in 200 solution of fixanol in water and dried without rinsing. The bactericidal effect of different concentrations was tested, the blankets being shaken over blood-agar plates. With this method of culture the number of particles released varies according to the state of the blanket, but it parallels roughly the dispersal of particles in bed-making. Ludlam's medium (Ludlam, 1949) and Chapman's salt agar (Chapman, 1945, 1949) were tried as selective media for staphylococci. The latter was found of some value in this

Blankets After Treatment with Various Concentrations of Fixanol

	Concentration of Fixanol in Dry Blanket				
	1 in 800	1 in 1,600	1 in 3,200	1 in 6,400	0
No. of colonies on agar	±	0	+	++	++++
Size of inhibition zones on staph. spread plate	++++	++	+++	+	0

FIG. 1.—Sterilization of blanket with fixanol C. Above: untreated. Below: treated.

work, but blood agar remained the principal medium. No growth was obtained with final dry concentrations up to 1 in 1,600, and at 1 in 6,400 growth was much reduced and no staphylococci were found (Table and Fig. 1).

The fixanol on the dried blanket coats the individual fibres and readily passes again into solution on contact with moisture. This can conveniently be demonstrated by shaking the treated blanket over a plate which has been freshly spread with *Staph. aureus* broth culture and dried. After incubation, as may be seen in Fig. 2, each fibre is surrounded by a narrow zone of inhibition where no growth has occurred.

FIG. 2.—Inhibition zones around wool fibres coated with fixanol C.

The concentration of fixanol forming in secretions that reach the blanket will depend upon the volume of secretion and the way it spreads amongst the fibres. A droplet of saliva on a treated blanket examined under a hand lens can be seen to spread to a limited extent (Fig. 3). It is reasonable to suppose that in such a droplet the strength of fixanol lies much above the minimum lethal concentration for staphylococci, which is 1 in 200,000, and even above that for the Gram-negative bacilli, 1 in 1,000. For routine use 1 in 6,400 dry content was chosen. As a check a 1 foot square (0.093 m.²) of blanket was heavily infected in a diluted broth culture of staphylococci, dried, and then washed with 16 other blankets in this chosen concentration. Shaken over blood agar before treatment, the square grew a shower of staphylococci, but afterwards none at all. Further, a blanket that had been treated was some weeks later infected with staphylococcus as above, dried, and shaken over agar. This also grew no staphylococci.

The same procedures were carried out with *Pseudomonas pyocyanea*, an organism which, with the exception of the tubercle bacillus, is probably the most resistant pathogen to this bactericide as to so many others. There was, as with the staphylococcus, complete failure to recover any of the test organisms when the blanket was infected before being treated with fixanol. When, however, a culture of *Ps. pyocyanea* was sprayed on to or soaked into a blanket square impregnated with fixanol in the usual strength, it was readily recovered later. The same applied even when the concentration of fixanol was raised four times. Evidently, in such absorbed fluids, the concentration of fixanol does not reach the lethal level for *Ps. pyocyanea*.

Thus, with this procedure the blanket leaves the laundry virtually sterile. Moreover, it will apparently retain, for a considerable period, sufficient material to destroy any staphylococci that may be picked up in secretions. The cost of the detergent is approximately 1d. a blanket, and the procedure is straight-

FIG. 3.—Saliva droplet adhering to wool fibres coated with fixanol.

forward. The impregnated blanket has a slightly smooth feel, it has no smell, and in routine hospital use for all blankets for a year there has been no indication of patients showing sensitivity to the treated blankets.

Discussion

The much higher frequency with which penicillin-resistant strains of staphylococci are recovered from hospital cases, as compared with those at home, is largely referable to cross-infection (Rountree and Thomson, 1949; Barber, Hayhoe, and Whitehead, 1949). Nasal carriers of such strains among hospital staff are also frequent, and there is a tendency for newly arrived nurses to acquire the prevailing phage type. Though staff may directly infect patients, both might, as postulated by the *Lancet* (1949), be infected by the hospital environment. The nose is an effective dust filter (StClair Thomson and Hewlett, 1896). This is by virtue of air turbulence and impaction of particles against the moist walls. Thus a ward nurse has something in common with a slit sampler.

It was of interest, therefore, to sterilize the hospital blankets for a period and to observe the effect on the incidence of carriers, and also of cross-infected wounds. Briefly, it may be reported that in three months there was no reduction in the carrier rate, while for wounds the small numbers left the matter in doubt, a certain amount of cross-infection still continuing. No doubt, as observed by Girdlestone, Bourdillon, and McFarlan (1951), there are a multiplicity of sources of infection. A wider trial is desirable in which the sterilization of clothes and other fabrics deserves consideration.

Whilst uncertainty remains on many points, it is not without importance that, as many have observed, pathogens are surviving for long periods in the woollen fabrics of our most immediate environment, and, moreover, that perhaps now this need no longer be so.

Summary

Cross-infection of wounds in a hospital has been investigated. Attention is directed to dust from blankets. From freshly laundered blankets *Staph. aureus* was frequently obtained, which organism readily survived the laundry process. During laundering bacteria pass from one blanket to the next.

Impregnation with cetyl pyridinium bromide (fixanol C) has been found a satisfactory way of sterilizing blankets. They remain, to a large extent, self-disinfecting.

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BACTERIOLOGY OF VAGINAL FLORA AFTER USE OF INTERNAL TAMPONS

BY

ELIZABETH N. BRAND, M.D., D.P.H.

The vaginal tampon is being used with increasing frequency for the absorption of the menstrual flow, but this subject has received little attention from the medical profession in this country, though there still may be a fair amount of prejudice against its use.

Correspondence for and against it appeared in the *British Medical Journal* (1938, 1939). For over 14 years Partridge (1939) had advised its use amongst her patients without there being any complaints or untoward results. Barton (1942) reported on the advantages of intravaginal tampons as menstrual guards.

The matter has been investigated fully by gynaecologists in the U.S.A. Sackren (1939) carried out an investigation on 21 patients and found no irritation in the vaginal or cervical tissues after the use of internal tampons. Thornton (1943) investigated 110 subjects for one to two years and arrived at the same conclusion. Karnaky (1941, 1943) claimed beneficial results in cases of sterility. Magid and Geiger (1942) investigated the bacterial flora in the vagina and cervix after the use of tampons in 25 women, over two menstrual periods,