THE VALUE OF ANTISEPTICS AS PROPHYLACTIC APPLICATIONS TO RECENT WOUNDS

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There have been groups of observers both in this country and in Germany who have been entirely sceptical about the value of the prophylactic application of antiseptics to wounds and who seem to incline to the opinion that such medication is likely to be more harmful than useful; e.g. Colebrook in a special report to the Medical Research Council (1928) stated that 'the ordinary "antiseptics" have a higher affinity for the leucocytes than for the microbes, and by combining with the former forfeit their microbicidal potential'. Further, he stated that with the exception of optochin and neosalvarsan, substances which have been tried as antiseptics are so rapidly fixed on serum proteins, blood cells, and the fixed tissues, that they fail to impart bactericidal power to the serum. Fleming (1924, 1931) in a number of ingenious experiments showed that injury to leucocytes is the most likely outcome of antiseptic applications to wounds, and has argued that they are more likely to be harmful than useful for that reaon; later (1938), he pointed out the superiority of the sulphonamides to the older antiseptics as tested by his 'slide cell' technique and in penicillin he has discovered an antiseptic which in respect of a high ratio of bactericidal potency to its toxicity to mammalian tissues approaches the ideal. This has given the clinical results which would be expected on theoretical grounds. There are, however, limitations to the use of penicillin as a general prophylactic antiseptic application which depend on (*a*) its failure to destroy some types of bacteria which cause suppuration, (*b*) its relative lability, (*c*) its present cost. There is therefore ground for further careful assessment of the best prophylactic antiseptic. Fleming (1940) has in fact admitted that there may be a place for proflavine in the interim dressing of wounds pending surgical treatment. The German opinion on the question of the prophylactic use of antiseptics seems to have been considerably influenced by the observations of Schimmelbusch, who had demonstrated that mice inoculated on a scarified area of the tail with anthrax bacilli could not be saved when the tail was amputated at a point proximal to the site of inoculation even 10 min. after the wound had been infected. It was concluded on this account that the penetration of bacteria from a wound into the deeper tissues was so rapid that local treatment of the wound was obviously futile. Schiemann & Wreschner (1922) and Weise (1922), however, who record these observations of Schimmelbusch, made similar observations with streptococci but showed that with this micro-organism not only tail amputation but also local application of antiseptics was often successful in saving mice so infected. They did not dispute the rapid penetration of the bacteria but concluded that in the case of the streptococcus the body could deal with limited numbers of these micro-organisms, if the local lesions from which they were reaching the general circulation were eliminated. Further, Browning (1943) in a discussion of the antiseptic action of the amino acridine compounds made an extensive survey of the literature on the influence of these substances on leucocytes and concluded that in view of the wide variations in results the particular technique adopted must be an important factor. Hence he suggests there is need for much caution in accepting such results as an indication of what will actually happen in the animal body.

In 1940 Garrod in a very illuminating review of the whole subject of antiseptics in wounds, stated a strong case for discrimination between the prophylactic use of antiseptics in wounds and the treatment of established infection of wounds. In the course of this review, in which he stressed particularly the claims of proflavine, he suggested that the less caustic of the phenolic disinfectants such as 'Cyllin' and 'Izal', which consist of emulsions of certain of the higher boiling homologues of phenol, and products such as 'Dettol' in which a cresol or xylenol has had its bactericidal power reinforced by halogenation, have a claim for consideration in the treatment of wounds. Interest in the prophylactic treatment of wounds by antiseptics was renewed by the introduction of the sulphonamides, which, unlike any other substances employed previously, had been proved effective in the treatment of established infection by several types of bacteria. These have also been much recommended and used in the prophylaxis of

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infection of war wounds, and advantage has been taken of their limited toxicity to apply them in the form of powder, which has been shown by Botterel, Carmichael & Cone (1941) to produce very little damage even when introduced experimentally in relatively large amounts to wounds of the brain. This conclusion has also been established in careful experimental work by Russell & Falconer (1940–1, 1943) and Russell & Beck (1944), who also note that the admixture of even 1 % of proflavine to the powder makes it much more irritant. The use of sulphonamides in wounds in the form of powder was first adopted by Jensen, Johnsrud & Nelson (1939) and probably first suggested for war wounds by Legroux (1940).

It has been widely adopted and favourable reports on experimental results are recorded, amongst others, by Bonnin & Fenner (1941), Bisgard & Baker (1942), McSwain & Glenn (1942), Colebrook (1940–1), Colebrook & Francis (1941), Key & Burford (1941), Baker (1942). Schneider (1941) and Goldberger (1942) strike an original note in recommending the combination of hydrogen peroxide solutions and sulphanilamide powder for wound treatment.

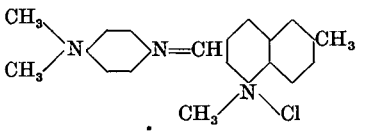
It seems altogether probable that in the case of infections by streptococci, bacteria which can be controlled by sulphonamides when they have succeeded in invading the body generally, the effect of local application of these drugs should be superior to that of most other known antiseptics. That they are in fact capable when given by mouth of controlling local streptococcal lesions tending to generalization with fatal issue, was of course demonstrated by Berger (1937) and others for prontosil. The same is not necessarily true of other bacteria such as staphylococci and anaerobes which are much less sensitive to sulphonamides. In fact, under the experimental conditions adopted by McIntosh & Selbie (1942, 1943), it has been found that even with sulphathiazole, in some ways the most effective of the sulphonamide group, the results with *Cl. welchii* and *Cl. novyi* infections are inferior to those obtained with proflavine, and Heggie & Heggie (1942) record that although local staphylococci infection of burns is not prevented by a full course of sulphapyridine by mouth, it is usually cleared up rapidly by the local use of lymphagogues followed by 0·1 % proflavine in buffered isotonic solution.

There appears, therefore, still to be ground for studying the best possible prophylactic treatment for all wounds. It was thought desirable to institute a comparison between sulphanilamide and a variety of other antiseptic substances in the prophylactic treatment of experimental wounds which were intentionally infected, since the earlier and extensive experimental work of this kind had mostly been executed before the sulphonamides were available.

It is clear that unless enormous numbers of animals are to be used it is not possible to get results which are statistically significant with a large number of different compounds; hence the range of antiseptics tested was inevitably restricted. Those examined were the following: Deosan, Dettol, Izal, Milton, tincture of iodine; a solution of equal parts of crystal violet and brilliant green, proflavine, quinanil; sulphanilamide solution and sulphanilamide powder, sulphathiazole. The investigation of Izal and Dettol seemed to be indicated in view of Garrod's suggestion quoted above, especially as we could find no record of investigation of these substances previously in experiments of this type. Tincture of iodine was included because of its wide use as a prophylactic antiseptic and because of its marked capacity for sterilizing the intact skin after infection with the more resistant spore-bearing bacteria (McLeod & Bevan-Brown, 1918). Milton and Deosan, which are solutions of sodium hypochlorite prepared by special methods, were included in view of the wide use of antiseptics of this kind, e.g. Dakin's solution, in the war of 1914–18. The mixed solutions of triphenylmethane dyes (first suggested to one of us by C. H. Browning) were included because of the remarkable potency of these compounds against the Gram-positive coccal forms and also because of long personal experience of their value in everyday use as a prophylactic antiseptic application to minor wounds. Iodine was the only antiseptic of those tried by McLeod & Bevan-Brown which approached this dye mixture in efficiency in killing off sporing aerobes on the surface of the skin. It has in any case had wide use in surface disinfection under the name of Bonney's paint (Bonney & Browning, 1918). It was obvious that one of the acridine dyes should be included in such a series, since so many records are extant of their ability to terminate experimental infections in animals which proved fatal in all controls or in a very high percentage of the controls.

Without attempting an exhaustive list of these the following may be quoted: Browning & Gulbransen (1925) on the control of diphtheria infection of wounds; Browning, Cohen, Ellingworth & Gulbransen (1931) on the control of intraperitoneal streptococcal infection; Weise (1922) and Schiemann & Wreschner (1922) on the control of streptococcal infection of superficial wounds in mice; Reinhardt (1922) on the control of chicken cholera and pneumococcal infections of guinea-pigs and mice; and Schiemann (1922), who achieved more limited success in the control of a much wider range of intraperitoneal infections of mice, a proportion of the animals being saved from infections with the pneumococcus, the Bacillus of Friedlander, the

Pasteurella of hen cholera, *B. anthracis* and *B. melitensis*. Much of this work was done with acriflavine, but since this is a substance of varying composition as Garrod (1940) points out, and since proflavine, one of its components, is less toxic (Russell & Falconer, 1940–1) and also one of the most potently antiseptic amongst the acridine derivatives (Rubbo, Albert & Maxwell, 1942) it was chosen for our experimental work. In 1929 Armitage, Gordon, Cohen & Ellingworth described observations on the value *in vivo* of 'quinanil', a new form of dye derived by sulphonation from a compound 2 (*p*-dimethylaminoanil) 6 methylquinolinemethochloride



described previously by Browning *et al.* (1926). This substance has been used by Boxall, Happold & Lloyd (1934) to purify cultures of protozoa from bacterial contaminants. Further closely related compounds such as 2(*p*-dimethylaminoanil) 6 methylallylacetylaminoquinolinemethochloride had been shown by Browning *et al.* (1931) to be very effective in controlling peritoneal streptococcal infection of mice and at least equal in this respect to acriflavine. There were, therefore, sufficient grounds for including quinanil in the group of antiseptics for comparative test. Sulphanilamide and sulphathiazole were the drugs chosen from the sulphonamide group.

PRELIMINARY EXPERIMENTS ON THE EFFECTIVENESS OF VARIOUS ANTISEPTICS IN MEDIA CONTAINING A CONSIDERABLE PROPORTION OF BLOOD

These investigations worked out much along the lines which were anticipated and will therefore only be summarized briefly. Four varieties of bacteria were used, a pyogenic staphylococcus, a haemolytic streptococcus (Lancefield group A), *B. coli* and *Cl. sporogenes*. Various concentrations of the antiseptics under investigation were allowed to act (*a*) on infected broth containing 30% of citrated human blood, (*b*) on infected and inspissated blood. Times of survival were determined up to 24 hr. by subculture. A small inoculum from a very young but actively growing culture was used in each case. Thus tested, the antiseptics could be roughly divided into three classes: (1) The sulphonamides, relatively impotent against all these bacteria when tried out *in vitro* in this way. (2) The antiseptics which appeared to act as general protoplasmic poisons, since all four bacteria were about equally sensitive to any one of them. This group included carbolic acid, Dettol, Izal, Lysol and Milton, which were active within the range of dilutions from 1/50 to 1/500. Of these Izal was the most powerful, and Milton the weakest. (3) The dyes—brilliant green, crystal violet, proflavine and quinanil. The notable point about these was the wide range in effectiveness for the coccal forms on the one hand, 1/10,000 to 1/100,000 concentrations proving bactericidal, and for the bacillary forms on the other, for which their effective concentration varied from 1/1000 to 1/10,000. Quinanil was peculiar in its high bactericidal potency for *B. coli*.

In general the titres obtained in the experiments with infected and dried blood were lower than those observed in blood broth.

In vivo *experiments*

(1) *Experiments on the control of mixed infections including anaerobes introduced to injured muscles*

This type of experiment was adopted because it seemed to us to correspond more closely in some respects to what happens in accidental injuries and those due to high explosives than do the numerous recorded experiments with pure cultures of anaerobes, washed spores, etc. Six experiments were done on guinea-pigs and one on rabbits. Various mixtures of anaerobic and aerobic bacteria commonly found in wounds were used in the different experiments. One anaerobic and two aerobic forms or two anaerobic and one aerobic were used in each experiment and a considerable series of antiseptics was tried out by injections to the injured and infected muscles, successive injections being made 1, 2 and 4 hr. after infection. Three animals were used for each antiseptic in every experiment.

It is clear from these results that one cannot depend on any of the substances used for the effective control of infections of this kind, and in this respect they reinforce the conclusion already reached in earlier experimental work by Gordon & McLeod (1941) on experimental anaerobic infections, that the prophylactic use of anti-gas-gangrene serum is more important than that of most bactericidal and bacteriostatic substances. Penicillin and some of the more recent sulphonamide preparations, such as marfanil (Evans, Fuller & Walker, 1944), have not been tried in experiments such as are described above, so far as we know.

Since survivals were so rare in these experiments, we did not consider that much was likely to be obtained from extending them. In so far, however, as they go, they have been analysed in Table 1 for such evidence of the comparative prophylactic and therapeutic value of the various substances tested as may be deduced from a limited investigation. These results are summarized by saying that in closed infections of these types only sulphathiazole gave more survivals than the controls and that the acridine and triphenylmethane dyes occasionally

Table 1

Animal and infection used in experiments:

Exp. I. Guinea-pig: *Cl. novyi, B. coli, Staphylococcus.*
Exp. II. As Exp. I.
Exp. III. As Exp. I.
Exp. IV. Guinea-pig: *Cl. septique, Cl. histolyticum, Streptococcus.*
Exp. V. Rabbit: *Cl. septique, Cl. sporogenes, Staphylococcus.*
Exp. VI. Guinea-pig: *Cl. welchii, Cl. sporogenes, Staphylococcus.*
Exp. VII. Guinea-pig: *Cl. welchii, B. coli, Streptococcus.*

Class of antiseptic	Antiseptic	Form and strength of application	Exp. I	Exp. II	Exp. III	Exp. IV	Exp. V	Exp. VI	Exp. VII	Total exp. animals	Survivals and delayed deaths
Sulphonamides	Sulphathiazole	Powder 0·19 g.	0/3	0/3	—	0/3	2/3 s.	3/3 s.	1/3 D.D.	18	5 s., 1 D.D.
	Sulphapyridine	Powder 0·1 g.	0/3	—	—	—	1/3 s. 2/3 D.D.	1/3 s. 1/3 D.D.	—	9	2 s., 2 D.D.
Dyes	Brilliant green and crystal violet	Solution 0·1–0·2 %	1/3 s.	0/3	1/3 D.D.	0/3	1/3 s.	0/3	2/3 D.D.	21	2 s., 3 D.D.
	Proflavine	Solution 0·1–0·2 %	1/3 D.D.	0/3	0/3	0/3	0/3	1/3 s. 1/3 D.D.	1/3 s. 1/3 D.D.	21	2 s., 3 D.D.
	Quinanil	Solution 0·08–0·1 %	0/3*	0/3	—	0/3	0/3	0/3	—	15	0 s., 0 D.D.
Phenolic	Dettol	Solution 1–5 %	0/3	0/3	0/3	0/3	1/3 s.	1/3 s. 2/3 D.D.	—	18	2 s., 2 D.D.
	Izal	Solution 1–5 %	0/3	0/3	0/3	0/3	1/3 s.	1/3 D.D.	—	18	1 s., 1 D.D.
Halogen	Deosan	Solution 5–25 %	0/3	0/3	0/3	0/3	0/3	1/3 s.	—	18	1 s., 0 D.D.
	Iodine in aqueous KI solution	Solution 0·5–1 %	0/3	0/3	—	0/3	0/3	1/3 D.D.	—	15	0 s., 1 D.D.
	Controls	Nil	0/3	0/3	0/3	0/3	1/2 s.	1/2 s.	0/4	20	2 s. —

1/3 s. = one of three experimental animals survived.
1/3 D.D. = one of three experimental animals died 24 hr. or more later than the longest surviving controls.
0/3 = no survival and no animal lived significantly longer than controls.
— = no experiment.

In each experiment an uninoculated animal received the same amount of the medicament as the experimental animals. In Exps. II, III and IV with brilliant green and crystal violet, and in Exp. II with proflavine, this guinea-pig died late.

delayed the fatal result. A closed wound with muscle injury and a copious mixed infection is, of course, a very exacting test, and there is a considerable possible field for prophylactic antiseptic application in lesser and superficial wounds. We turned, therefore, to the established technique of trying out a range of substances on superficial wounds of the mouse infected with streptococci.

(2) *Experiments on the control of streptococcal infections of superficial wounds in the mouse*

Of those readily available, the streptococcal strain Richards killed mice most regularly after infection of superficial wounds. This strain is of course well known as a sulphonamide-sensitive strain and was therefore one likely to give good results with these compounds.

In all, nineteen experiments were performed, each involving the use of fifty to sixty mice. The method of wounding was to pick up the loose skin of the mouse's back and snip through it with scissors so as to leave a lozenge-shaped exposure of subcutaneous tissue varying from 1 × 1 cm. to 2 × 2 cm. In about half the experiments the base of the wound was scarified before inoculation to allow of more rapid penetration of the streptococci. One to three drops of an 18 hr. 10% serum broth culture of the streptococcus were dropped on to the exposed tissue immediately after wounding and treatment by dropping on 0·25 c.c. of the antiseptic solution was carried out 15 min. after wounding, again at 3 hr. and next morning.

In each of the first seventeen experiments, all or most of the antiseptics were used, five mice being allotted to each. The expected superiority of sulphanilamide in powder form having by then been sufficiently established, three more experiments were carried out in which the two most promising of the other antiseptics—proflavine and the triphenylmethane dye mixture—were compared with Izal, using larger numbers of mice for each antiseptic and for the control, the object being to establish more clearly the relative merits of the three antiseptics and to determine more certainly by larger control groups whether the prophylactic use of antiseptics was in fact justified.

These results are presented in two tables: Table 2 for experiments without scarification, Table 3 for those with scarification of the base of the superficial wounds.

The antiseptics used in these experiments were the same as in the previous one with mixed infections, with the exceptions that Milton was substituted for Deosan and that several dilutions of most of the antiseptics were tried. Also a comparison was made between sulphanilamide powder and a concentrated solution of sulphanilamide and the dyes were used in the form of powder in some experiments. The fate of the animals was always followed for 4 weeks and in all cases of late deaths the fact of death by streptococcal septicaemia was verified by inoculating the heart blood or the cut surface of the spleen to fresh blood agar. The appearance of numerous colonies of characteristic appearance and with well-marked haemolytic areolae was demonstrated in this way in late deaths up till the end of the 4th week. In the last two experiments streptococcal infection was established in the same way in every animal that died. The survivors from the last experiment were kept under observation for 8 weeks. There were one or two deaths after the 6th week but no streptococci were obtained in cultures of the spleen of these animals. These last have been recorded as recoveries.

In view of the facts that there were one or two experiments in which considerable numbers of survivals were recorded with a variety of antiseptics and others in which no success was recorded with any antiseptic, it is clear that in spite of endeavours to avoid it, there was a fluctuation in virulence of the inoculum from experiment to experiment. Since, further, some antiseptics were omitted from certain of the experiments an adequate comparison of the value of the different substances could not be made on the ground of these experiments.

A careful appraisal of these experiments therefore led to the conclusion that they could only be considered as giving some broad general indications on the relative merits of the different antiseptics and guide us in the selection of one or two substances to be tried out in further series of experiments so planned as to yield reliable statistical evidence of the absolute value of prophylactic antiseptic treatment of wounds and of the comparative merits of the substances chosen.

Further, a point rightly raised in the discussion of these results was that in the antiseptic treatment used in most cases the wounds underwent a lavage which the control wounds did not have. This objection was eliminated in the later experiments. The broad conclusions suggested by the results recorded in these tables are: (1) That the experiments with scarification were a much more severe test, the percentage of successful results being from two to five times lower with every antiseptic tried and the survival of controls being less than 2%. (2) That the more concentrated the antiseptic the better the results—thus the only considerable number of successful results in the scarified animals was obtained with sulphanilamide or the dyes in powder form. An exception was quinanil, which gave the most promising results in 0·1% dilution but was too toxic when applied as a powder. This was further borne out in the experiments without scarification, in which the stronger dye solutions gave much better results than the weaker ones.

(3) That with every antiseptic tried, excepting a weak aqueous solution of iodine, there was some indication of advantage in antiseptic treatment and certainly none of the promotion of bacterial invasion.

superiority of the dyes to some of the widely used phenolic antiseptics suggested in the earlier experiments be confirmed?

Three further experiments were carried out, in which the numbers of animals in each group were

Table 2. *Experiments without scarification of base of wound*

Class of antiseptic	Treatment	No. of experiments with 5 mice	10 mice	20 mice	Total mice	Survivals	Delayed deaths
Sulphonamides	Sulphanilamide powder	4	—	—	20	10	9
	Sulphanilamide solution 2%	2	—	—	10	3	1
Dyes	B.G. and C.V. solution 2% (of each)	—	2	—	20	10	—
	B.G. and C.V. solution 0·5%	7	—	—	35	6	6
	Proflavine solution 1% or 0·5%	—	3	—	30	16	5
	Proflavine solution 0·1%	7	—	—	35	8	8
	Quinanil solution 0·1%	7	—	—	35	9	4
Phenolic	Dettol solution 5%	7	—	—	35	10	1
	Izal 2%	7	3	—	65	9	1
Halogen	Milton 10%	7	—	—	35	7	5
	Iodine B.P. tincture	4	—	—	20	9	2
	Iodine 0·3% in aqueous KI solution	3	—	—	15	0	5
	Nil. (Controls)	7	—	3	95	5	—

Table 3. *Experiments with scarification of base of wound*

Class of antiseptic	Treatment	No. of experiments with 5 mice	10 mice	Total mice	Survivals	Delayed deaths
Sulphonamides	Sulphanilamide powder 0·5 g.	—	6	60	17	28
	Sulphanilamide 2%	3	4	55	0	9
Dyes	B.G. and C.V. powder	1	2	25	5	2
	B.G. and C.V. 0·5% solution	5	—	25	2	3
	Proflavine powder	1	2	25	6	3
	Proflavine 0·1 and 1% solution	5	1	35	3	2
	Quinanil powder	—	2	20	1	—
	Quinanil solution 0·1%	5	—	25	3	4
Phenolic	Dettol 5%	5	—	25	1	1
	Izal 2% solution	7	1	45	2	—
Halogen	Milton 10%	5	—	25	1	1
	Iodine B.P. tincture	7	—	35	3	—
	Nil. (Controls)	8	1 (20 mice)	60	1	—

By delayed deaths is meant death 3 or more days later than the longest lived of the controls in the particular experiment. The average length of survival in days of the controls over the whole nine experiments (60 mice) was 4 days.

Taking these observations into account, therefore, further series of experiments were planned with a view to obtaining data suitable for establishing statistical evidence. These were directed to answering two questions: (*a*) Would lavage with antiseptic dyes save significantly more wounded and infected mice than washing with water? (*b*) Would the

larger and the numbers of antiseptics tested were smaller.

They were limited to proflavine and the triphenylmethane dyes, the most effective antiseptics in the earlier experiments, and to a few well-known examples of the phenolic group. Penicillin was tried in one experiment on account of the general interest

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of the comparison, although it is not upon its local application as a prophylactic antiseptic that its great value depends—ample evidence of its value in prophylaxis when given parenterally was obtained from experience with wounds in the B.L.A. (Porritt *et al.* 1945).

In the first series five experiments were done with batches of 100–120 mice. In each, one lot of forty mice which were wounded, inoculated with streptococcus and treated by washing with water was included, and also another lot in which the mice were washed with a solution of brilliant green and crystal violet after wounding and inoculation; the remaining lots were made up in a variety of ways. The series is therefore given in detail in Table 4.

This series of experiments fell into line with the earlier ones; the survivals in the controls were higher but so also were those amongst the treated animals, there was no evidence that cleansing by washing had any importance, and the value of proflavine and the triphenylmethane dyes was very clearly proved.

Two further experiments, each on 500 mice, were planned to test more carefully the relative values of some of the better known and most widely used phenolic antiseptics and the dye antiseptics mentioned. In one of these penicillin was included. These experiments were designed to obtain reliable figures despite the fluctuations in virulence of the culture. As each of these series consisted of five identical experiments they have been summarized without giving so much detail. In view of the fact that in all previous experiments the great majority of the mice died in the first week it was decided to close the experiments after 7 days.

The results are given in Tables 6 and 7.

The mice were actually observed up to 10 days but the results recorded at the later day only increased the evidence for the superiority of the dyes and brought Lysol and Dettol more nearly to the same level.

It is to be noted that a certain amount of Salmonella infection appeared as a complication in the first of these experiments, but the same general result was obtained notwithstanding.

Table 4. *First series of* 500 *mice* (*observed for* 4 *weeks after infection*)

	No. of mice	Infection	Treatment	Survivals	% survivals
Exp. I	20	None	Wound, sterile medium, washed water	20	100
	40	*Streptococcus*	Wound, washed water	8	20
	40	*Streptococcus*	Wound, washed B.G. and C.V.	17 (+2°)	42·5
		Streptococcus + water *v.* *Strept.* + dyes $\chi^2 = 4{\cdot}46$, $P < 0{\cdot}05$.			
Exp. II	20	None	Wound, washed B.G. and C.V.	14	70
	40	*Streptococcus*	Wound, washed water	4	10
	40	*Streptococcus*	Wound, washed B.G. and C.V.	18	45
		Streptococcus + water *v.* *Strept.* + dyes $\chi^2 = 12{\cdot}3$, $P < 0{\cdot}01$.			
Exp. III	40	*Streptococcus*	Wound, washed water	1	2·5
	40	*Streptococcus*	Wound, washed 0·33 % Izal	0	0
	40	*Streptococcus*	Wound, washed B.G. and C.V.	11	27·5
		Streptococcus + water *v.* *Strept.* + dyes $\chi^2 = 9{\cdot}8$, $P < 0{\cdot}01$.			
Exp. IV	40	*Streptococcus*	Wound, washed water	6	15
	40	*Streptococcus*	Wound, washed 1 % proflavine	19	47·5
	40	*Streptococcus*	Wound, washed B.G. and C.V.	24 (+1°)	60
		Streptococcus + water *v.* *Strept.* + proflavine $\chi^2 = 9{\cdot}8$, $P < 0{\cdot}01$.			
		Streptococcus + B.G. and C.V. *v.* *Strept.* + proflavine $\chi^2 = 1{\cdot}23$, $P < 0{\cdot}2$.			
Exp. V	40	*Streptococcus*	Wound, washed water	3	7·5
	40	*Streptococcus*	Wound, washed 1 % proflavine	21 (+5°)	52·5
	40	*Streptococcus*	Wound, washed B.G. and C.V.	14 (+3°)	35
		Streptococcus + B.G. and C.V. *v.* *Strept.* + proflavine $\chi^2 = 2{\cdot}5$, $P < 0{\cdot}1$.			

The figures +2°, etc. in parentheses in the survival column indicate the number of mice which died without evidence of streptococcal infection.

P indicates probability referred to unity as a certainty.

$< 0{\cdot}01$ indicates the found value of χ^2 would only be so high once in more than a 100 times by chance, i.e. it may be assumed that some real difference between the treatments exists.

$< 0{\cdot}2$ indicates that the found value of χ^2 would be so high once in less than five times and, therefore, no difference in treatments can be assumed.

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Table 5. *Extended control of toxicity of dye preparations*

Exp. VI. Observations for 10 days only.

No. of mice	Infection	Wounded	Treatment	Survivals
20	Nil	+	Nil	20
20	Nil	+	Washed with 0·25 % proflavine	20
20	Nil	+	Washed with 1 % proflavine	19 (one mouse died on 7th day)
20	Nil	+	Washed with 0·5 % crystal violet and brilliant green	19 (one mouse died on 9th day)
20	Nil	+	Washed with 2 % crystal violet and brilliant green	18 (2 mice died, one on 8th and one on 9th day)

Table 6. *Second series of* 500 *mice* (*observed for* 1 *week*)

Survivals after 7 days in groups of twenty mice all wounded and washed with water or antiseptic solution

Exp.	Non-infected, washed water	Infected, washed water	Infected, washed B.G. and C.V. 2 %	Infected, washed Lysol 2 %	Infected, washed penicillin 1000 U/c.c.
I	17 (+3°)	4	16	12	15
II	20	5	14	8	9 (+1°)
III	20	13	20	15	20
IV	18 (+2^s)	9 (+1° and 1^s)	17 (+3^s)	14 (+2° and 2^s)	16 (+4^s)
V	19 (+1^s)	19	20	18 (+1°)	19 (+1^s)

Table 7. *Third series of* 500 *mice* (*observed for* 1 *week*)

Survivals after 7 days in groups of twenty mice all wounded and infected, but washed variously with water and antiseptic solutions

Exp.	Washed water	Washed 2 % Lysol	Washed 2 % B.G. and C.V.	Washed 10 % Dettol	Washed undiluted Dettol
I	9	8 (+1^s and 1°)	17	6	8 (+2°)
II	3	3	18	9	4 (+1°)
III	5	13	15	10	5
IV	5	7	15	4	7
V	4	4	16	10	6 (+1°)

The figures in brackets, 1°, etc. indicate how many there were amongst the animals which died from which no streptococci were recovered at post-mortem; the figures 1^s etc. indicate how many there were amongst the animals which died from which a Salmonella was recovered at post-mortem and which was probably responsible for the death of the animal.

Experiments summarized in Tables 6 and 7 were planned on a 5 × 5 Latin square principle—each cell of twenty mice—the three factors being 'treatment', 'time of experiments' and 'order' in experiments.

The second factor was intended to be a control on any change in virulence of culture during the whole period of the experiment; the third factor was to control any difference due to fatigue on the part of the experimenter in handling a series of 100 mice in one operation.

The analysis of variance is of interest if the number of survivors of the twenty mice in each cell is taken as a basis for an estimate.

In Table 6 the difference due to 'treatment' is highly significant and the effect of 'time' of some significance. 'Order' had no effect.

The residual mean square was found to be 6·6 at 16 degrees of freedom.

In Table 7 while 'treatment' was highly significant, neither 'order' nor 'time' had a significant effect.

Nevertheless, the residual mean square (random error variance) amounted to 6·3 at 20 D.F. Whether accidental or not a residual variance of less than 7, say S.D. of 2·6 in twenty-five groups of twenty mice in two different tests, is an indication of unusual uniformity.

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GENERAL DISCUSSION

Over all these experiments there is a possible superiority of proflavine to the brilliant green and crystal violet mixture, although this does not appear in every experiment and there is also some evidence that with a large wound relatively to the total body surface the latter used as powder or in highly concentrated solutions may prove toxic. This conclusion is suggested by Exp. II in Table 4, but as this experiment lacked a group of animals wounded but neither treated nor infected, a further experiment dealing more carefully with the question of dye toxicity was carried out. This is recorded in Table 5. There is no clear evidence from this observation that proflavine was toxic in the concentrations used; but there was a suggestion that the solution of crystal violet and brilliant green was too concentrated.

On this score quinanil also seems to be excluded as the best prophylactic antiseptic, and undiluted Dettol, which has sometimes been recommended, has given worse results than a 10% solution.

The sulphanilamides, which are relatively devoid of toxic effect and when used as powders are shown to give results in streptococcal and anaerobic infections which are equal to or better than those obtained with the dyes, are excluded as general antiseptics on account of their relative failure to control some pathogenic bacterial forms, especially the coliform bacteria and the staphylococci.

It is interesting that although the results obtained with acridine dyes were the best apart from the sulphonamides, they did not, in view of the survival of more than 1% of controls in every group of experiments or large single experiment, compare favourably with those recorded for such dyes by Collier & Bernhagen (1928–9), who, notwithstanding the deaths of all controls, obtained 88% of survivals in one series and 43% in another; or by Schiemann & Wreschner (1922), who obtained 40% survivals in the same circumstances; while Weise (1922) with only one death of a control (1·7%) obtained 69% survivals with trypaflavine (acriflavine) and 66% with rivanol.

The discrepancy may be explained by the fact that these German observers were using different strains of streptococci, possibly by differences in the mice (our experiments were not restricted to white mice as were most of theirs). The fact that we used more controls may be partly responsible. A possible superiority of the acridine compounds used in these earlier experiments cannot be excluded.

Rubbo *et al.* (1942) drew attention to the antiseptic activity of the 5-mono-amino-acridine compound which is greater than that of proflavine, a 2-8-diamino acridine; and Albert, Francis, Garrod & Linnell (1938) have shown that in certain cultural conditions both 2-5-diamino-acridine-mono-hydrochloride and acriflavine are more actively bactericidal than proflavine. Proflavine has the advantage over acriflavine of lesser toxicity to mice and of more constant composition, but it is not impossible that certain products elaborated under the latter name are more effective in the experimental conditions described. In any case there seems to be ground for considering the question of the relative merits of the sulphonamides and flavines for *local* applications in streptococcal infections as one which is still open. When, however, the greater antiseptic powers of the acridines for such bacteria as the staphylococci are also taken into consideration, it is clear that there is much justification for the recent tendency to emphasize the need for restoring these dyes to an important place in the prophylactic treatment of wounds as suggested in the work of Mitchell & Buttle (1942) and so long advocated by Browning and his collaborators and by Garrod. In doing so account will have to be taken of the careful experimental work of Russell and her collaborators, Russell & Falconer (1940–1, 1943); Russell & Beck (1944), on the toxicity of various antiseptics to delicate tissues especially the brain. These emphasize the fact that proflavine, 2.7-diamino-acridine, and 5 amino-acridine-hydrochloride in 0·1% buffered solutions in isotonic saline do not produce demonstrable damage to tissues. In this respect they differ from acriflavine and all other antiseptics which they tested, except the sulphonamides. They also established that in contact with muscle or brain the acridines mentioned above in powder form or as dilute mixtures with sulphonamide powders produced tissue necrosis not caused by the sulphonamide powder alone. Stronger solutions of proflavine (1%) such as have been found effective in control of infection in our experiments are not considered in the published work of Russell and her collaborators.

CONCLUSIONS

1. That a considerable series of experimental investigations of the early application of antiseptics to severe superficial wounds heavily infected with a virulent streptococcus indicates that prophylactic treatment of such wounds with antiseptics is justified.

2. That this is true of a wide range of well-known antiseptics; but that the results with sulphanilamide powder, proflavine and triphenylmethane dyes are outstanding.

3. That the various well-known phenolic antiseptics tested on infected wounds did not give results equal to those just cited.

4. That for the streptococcus used in this series of investigations the best results were obtained

with sulphanilamide powder, but that proflavine and the brilliant green-crystal violet mixture which came very close to the sulphanilamide powder in these experiments are probably in view of their wider range of antiseptic activity the best 'first-aid' application to wounds at present available.

5. That for wounds involving extensive and deep damage to the tissues and multiple infection including anaerobes, the results are not promising with any of the antiseptics investigated.

6. That the failure to apply the best general antiseptic in suitably diluted solutions to recent and open wounds in danger of infection is a grave mistake *and that the preparation of the skin for surgical operations with such antiseptics would probably lead to the elimination of some cases of post-operative sepsis. These still occur too frequently.*

7. That these recommendations do not apply to very extensive injuries in which intra-muscular penicillin, as used with brilliant results in the B.L.A., is indicated, since toxic effects might arise from absorption of dye over a very wide area.

8. That when capacity to sterilize all of the following: streptococcus, staphylococcus, *B. coli* and *Cl. sporogenes* in media rich in blood or in inspissated blood was tested with the antiseptics used, proflavine was the best, quinanil, the triphenylmethane dyes and Izal following in that order; quinanil was, however, much the most active against *B. coli*. Such tests, while probably a useful guide to the disinfectant value of these products *in vitro* in the presence of blood, cannot serve as an indication of the probable value of antiseptics in wounds, even when used for prophylactic purposes.

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