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Skin disinfection

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Although skin disinfection has been a subject of interest and research for over 100 years, there is no generally accepted procedure for use either at the operation site or on the hands of surgeons and nurses. This state of affairs is understandable, for new antiseptics continue to appear, and the information which clinicians have had from laboratory study on their relative and absolute merits is far from clear. Depending on whether they were testing the effects of antiseptics on the superficial or the deeper flora of the skin, bacteriologists have stressed either the 'virtual disinfection' by some antiseptics or the impossibility of removing more than a fraction of the skin flora without destroying the skin. Moreover, many discrepancies in the evaluation of individual antiseptics have been due to differences (and often deficiencies) in the technique of testing.

THE PURPOSES OF SKIN DISINFECTION

In hospital practice the removal of bacteria from the skin is desired essentially for two reasons: (1) To prevent cross infection by blocking the transfer of pathogenic bacteria from the hands of nurses and doctors to the susceptible tissues of patients; and (2) to prevent self-infection of patients by blocking the transfer of pathogens from the skin to the underlying tissues on a knife blade or a needle. In the case of the surgeon's or the nurse's hands, the object is to remove or reduce the numbers of pathogens on the surface of the skin, and to achieve a cumulative effect by repeated application of the antiseptic. At operation sites, on the other hand, it is desirable that pathogens should be removed from the deeper layers as well as from the surface of the skin, and often there will be no opportunity for more than one application of the antiseptic. In addition to the removal of vegetative organisms, preparation of the operation site and hand cleansing involve the removal from the exposed skin and from under finger nails of chemical dirt, grease, and the spores of pathogenic clostridia which cannot be destroyed by short exposure to any antiseptic. All these factors must be considered in defining the requirements for a technique of skin disinfection.

BACTERIAL FLORA OF THE SKIN

The healthy skin may be regarded as a selective medium. Certain pathogens, *e.g.*, *Streptococcus pyogenes*, are killed by unsaturated fatty acids of the sebaceous secretions, and others, such as the Gram-negative bacilli, are to a large extent killed when the fluid medium in which they were deposited on the skin evaporates (Burtenshaw, 1942; Ricketts, Squire, and Topley, 1951). The bacteria relatively unaffected by these factors which can multiply on the skin fall mainly into three groups: (1) Micrococci, (2) corynebacteria, and (3) *Propionobacterium acnes*, an anaerobe which Evans, Smith, Johnston, and Giblett (1950) have found to be the predominant organism (see also Pillsbury and Kligman, 1954). These groups of bacteria are the 'resident' flora defined by Price (1938, 1957) as the stable bacterial population of the skin which lives and multiplies there, in the depths and hair follicles as well as on the surface. Resident bacteria appear to be for the most part harmless commensals, but they sometimes include *Staphylococcus aureus*. What Price describes as the 'transient' flora includes any bacteria which are deposited on the skin from the environment; in hospitals these will include a variety of potential pathogens.

THE ASSESSMENT OF SKIN DISINFECTION

The methods used to assess the value of skin disinfection have been mainly concerned with four criteria: (1) The incidence of wound sepsis; (2) the removal of the superficial flora, represented by cultures deposited on the skin; (3) the removal of resident as well as transient flora, shown by bacterial counts in standard hand-scrubbing tests; and (4) the bacteria in skin biopsies taken after pre-operative disinfection.

The first of these criteria is clearly the most valuable when it can be applied successfully, but its scope has been very limited so far. The classic example is the demonstration by Semmelweiss in 1847 of a dramatic fall in the death rate from puerperal sepsis in maternity wards after the introduction of routine hand disinfection with chlorinated

lime by doctors and midwives (see Sinclair, 1909). A recent example is the fall in staphylococcal infection of the newborn following the introduction of hexachlorophene emulsion (Farquharson, Penny, Edwards, and Barr, 1952; Hill, Butler, and Laver, 1959). But when, as in these studies, the initial incidence of infection is small or highly variable, it is difficult to assess the value of skin disinfection by a fall in the sepsis rate. Clinical records of wound 'sepsis' are notoriously variable, and reports (*e.g.*, Kramer and Sedwitz, 1944) on the virtual elimination of sepsis in clean operation wounds following the introduction of a particular antiseptic provide little evidence of its value unless special techniques of recording are used.

Colebrook and Maxted (1933), Gardner and Seddon (1946), and Gardner (1948) allowed bacterial cultures to dry on the skin and then treated the inoculated areas with antiseptics; estimates were made of the bacteria which survived exposure to the antiseptics. Story (1952) developed this method, using a penicillin-resistant staphylococcus as the test organism and a selective medium containing penicillin. Bacteriostatic controls or neutralizers were used to avoid erroneous results through the transfer of antiseptic to culture plates. By the use of these methods of testing, 'virtual disinfection', *i.e.*, destruction of at least 99·9% of the organisms applied, was obtained in about 20 seconds when the surface was treated with 2% iodine in 70% ethyl alcohol. The effectiveness of an antiseptic was estimated by the time it took to achieve virtual disinfection, and by the proportion of samples in which this effect was obtained.

Price's test for the resident flora (Price, 1938, 1957) involves a standard one minute scrub with soap and water in a series of eight basins before and in a second series of eight basins after treatment with antiseptic followed by neutralizer. Simpler tests for the resident flora have been devised by Cade (1950), Quinn, Voss, and Whitehouse (1954), and Bowers (1950). Price (1951) introduced a 'spot testing' method to study the bacterial flora of different areas of the body. The proportion of resident bacteria shown in such tests to be removed by antiseptics falls considerably short of virtual disinfection; *e.g.*, after two minutes' application of 2% iodine in alcohol, there was a 95% reduction in the flora shown by the serial basin test.

The culture of skin biopsies taken after preoperative disinfection has been used for the assessment of skin antiseptics by Walter (1938), Gardner and Seddon (1946), Murphy, Dull, Gamble, Fultz, Kretzler, Ellis, Nichols, Kucharczuk, and Zintel (1951) and Myers, MacKenzie, and Ward (1956). This method has the virtue of providing a sample of bacteria from all levels of the skin, but it is awkward to use and for quantitative assessments should include the excision of skin from untreated as well as from disinfected areas. A surprisingly large proportion (23/24) of such biopsies was found to be free from detectable bacteria after four minutes' pre-operative treatment with 0·5% aqueous chlorhexidine (Myers *et al.*, 1956). This might cast some doubt on the view that the deeper layers of the skin retain their flora after disinfection; but as the biopsies were small fragments of tissue, some of which yielded no bacteria even before the antiseptic was applied, the organisms removed may in most cases have been superficial or transient flora, deeper resident bacteria being absent from the samples examined.

ANTISEPTICS USED FOR DISINFECTING THE SKIN

The chief properties required in an antiseptic for operation sites are (1) that it should be rapidly active against a wide range of microorganisms (ideally against all pathogens); (2) that it should kill the organisms and not merely inhibit their growth; and (3) that it should not damage the skin or the underlying tissues either by direct toxic action or by sensitization. Other properties, such as penetration and temporary staining of the skin to show the disinfected area, may be desirable. Antiseptics used for regular disinfection of the hands need not be as rapid in their action as those used on operation sites, and it is an advantage if they leave a film on the skin which continues to inhibit the growth of bacteria.

Of the wide range of antiseptics which have been used for skin disinfection, many do not fulfil these requirements. Ordinary soaps, which have a limited activity against some bacteria, are inactive against staphylococci (Bayliss, 1936). Phenol, which was used by Lister (1875) in a 5% aqueous solution, was later discarded because of its toxicity and because other antiseptics were active in much lower concentrations. Among the latter were many compounds of mercury, including mercurochrome, merthiolate, and the phenyl mercuric salts, but these antiseptics are predominantly bacteriostatic (Geppert, 1889; Hoyt, Fisk, and Burde, 1942). The great vogue of the mercurials has undoubtedly been stimulated by deceptive tests in which inhibitory concentrations of the antiseptic were carried over to culture plates. The acridine dyes (Albert, 1951) are strongly bacteriostatic, but their bactericidal action is slow and selective; the Gram-negative bacilli (in particular *Ps. pyocyanea*) are relatively insensitive to these compounds. Alcoholic solutions of acridines and mercurials are often used, but the former apparently owe the whole of their dis-

infectant powers to the solvent (Gardner and Seddon, 1946), and mercurials have been found actually to reduce the skin disinfecting powers of alcohol (Price, 1957). Colebrook and Maxted (1933) showed that chloroxylenol has outstanding merit as a hand disinfectant in midwifery because of its action against *Strep. pyogenes*, but this compound is deficient in activity against the Gram-negative bacilli, and in particular against *Ps. pyocyanea* (Lowbury, 1951).

Iodine was recommended by Senn as a skin disinfectant in 1905, and ethyl alcohol was used still earlier for this purpose (Epstein, 1897). Both have a rapid bactericidal action against a wide spectrum of vegetative organisms. Ethyl alcohol has optimal skin disinfecting power when diluted with water, preferably at concentrations between 70 and 90% (Epstein, 1897; Price, 1957). Colebrook and Maxted (1933) found an aqueous solution of iodine to be an effective bactericide for the hands with a prolonged action after drying. Unfortunately, iodine causes severe sensitivity reaction in some patients (*e.g.*, Murphy *et al.*, 1951; Zintel, 1956) and has for this reason been avoided by many surgeons. Recently, mixtures of iodine with certain surface active agents (iodophors) have been found to be free from the staining and sensitizing effects while retaining the disinfectant power of aqueous or alcoholic iodine (Gershenfeld and Witlin, 1955); of these antiseptics Virac has been specifically recommended for skin disinfection (Frisch, Davies, and Krippaehne, 1958).

The quarternary ammonium compounds have been a popular choice for skin disinfection because in addition to their disinfectant powers they are good detergents. Price (1950) found an additive effect of the two antiseptics when alcoholic solutions of benzalkonium chloride were used for skin disinfection. The value of aqueous solutions of such compounds is more doubtful. Like the mercurials, quaternary compounds are apt to give misleading results in tests of skin disinfection through the transfer to culture medium of bacteriostatic concentrations which do not kill bacteria (Neufeld, 1943). Moreover, a film of the antiseptic may be left on the skin concealing viable organisms (Miller, Abrams, Huber, and Klein, 1943). A potential disadvantage of these compounds is their incompatibility with soap, traces of which may be present on the skin and interfere with disinfection by a quarternary compound.

Of the antiseptics introduced in recent years two are of special interest for their role in skin disinfection. Hexachlorophene or G.11, a bis-phenol, was first described by Traub, Newhall, and Fuller (1944); its activity primarily against Gram-positive organisms, its compatibility with soap, its slow but cumulative action through deposition as a film on the skin, and its lack of toxic or sensitizing properties make it very suitable for regular use as a hand disinfectant by surgeons and nurses. At a concentration of 2% in bar soap, or at 3% in a cream containing an anionic detergent *p*HisoHex, hexachlorophene is found to be a useful agent for this purpose (Seastone, 1947; Hufnagel, Walter, and Howard, 1948; Smylie, Webster, and Bruce, 1959). Chlorhexidine (Hibitane) has been reported to have value, in aqueous or alcoholic solution, as an antiseptic for the operation field (Myers *et al.*, 1956), and also as a hand disinfectant applied in a cream by nurses (Murray and Calman, 1955; Calman and Murray, 1956).

At the Birmingham Accident Hospital we have recently investigated the relative merits of these and of some other antiseptics for the disinfection of hands and of operation sites (Lowbury and Lilly, 1960; Lowbury, Lilly, and Bull, 1960). The essentials of this study are summarized in the following sections.

THE SURGEON'S HANDS

Devenish and Miles (1939) found up to 24% of rubber gloves punctured at the end of operations. Large numbers of bacteria could easily be transferred to an operation wound through such holes and also through wet patches on the sleeves of theatre gowns.

We compared a number of alternative measures by which these hazards might be reduced (Table I). The tests were made on five members of the laboratory staff. After scrubbing up with soap and water (control series) or scrubbing up and, in addition, using some form of disinfection, these operators wore rubber gloves for an hour, one glove in each pair having a small pin hole at the tip of each finger. After this mock operation, the gloved hands were washed and dried, and samples were taken from which estimates were made of the bacteria that emerged through holes in the gloves and of the bacteria left inside the gloves after use. Appropriate neutralizers and bacteriostatic controls were used.

There was a substantial reduction in the numbers of bacteria escaping through holes in the gloves and deposited inside the gloves after the use of *p*HisoHex for the scrub and for all ablutions in the previous week, and a smaller reduction after a similar use of hexachlorophene soap. Very good results were also obtained after a three-minute rinse with 70% ethyl alcohol (slightly improved by the addition of 0·5% chlorhexidine digluconate), and by the addition to glove powder of neomycin and bacitracin (5 mg. per gram of each); a smaller effect

TABLE I

EFFECT OF ANTISEPTIC TREATMENT ON FLORA OF HANDS AFTER ONE HOUR'S OPERATION

Antiseptic Treatment of Hands	*Mean Bacterial Counts (per ml.) on Glove Washings after*			*Mean Estimated Counts (per ml.) on Washings of Bacteria Emerging through Holes in Gloves after*		
	(1) *Scrubbing up + Antiseptic Treatment* (10 *Samples*)	(2) *Scrubbing up Only (Controls)* (10 *Samples*)	(3) (1) *as %* *of* (2)	(1) *Scrubbing up + Antiseptic Treatment* (19-20 *Samples*)	(2) *Scrubbing up Only (Controls)* (18-20 *Samples*)	(3) (1) *as %* *of* (2)
Neomycin + bacitracin in glove powder	0·6 ± 1·1	332 ± 120	0·18	3·7 ± 3·1	73·6 ± 17	5·0
70% alcohol rinse (3 minutes)	3·0 ± 0·7	62·5 ± 18	4·8	7·5 ± 5·4	81·0 ± 12	9·2
70% alcohol with 0·5% chlorhexidine rinse (3 minutes)	3·1 ± 0·7	151 ± 48	2·1	3·25 ± 2·1	69·9 ± 14	4·6
Hexachlorophene soap (for 5-minute scrub and previous week)	14·9 ± 6	169 ± 66	8·8	7·3 ± 2·8	51·2 ± 8·7	14·3
*p*HisoHex (for two-minute scrub and previous week)	1·9 ± 0·7	127 ± 39	1·5	2·7 ± 1·3	63·8 ± 13	4·2
Spirit swab	79·5 ± 36	392 ± 140	20·3	24·2 ± 4·1	63·2 ± 14	38·3

was obtained after rapidly mopping and drying the hands with industrial spirit. In favour of hexachlorophene preparations are the facts that no additional time is needed for applying the antiseptic, and that the disinfection applies to the whole surface of the body; the risk of contamination through wet sleeves as well as through holes in gloves should therefore be reduced.

THE NURSE'S HANDS

For periods of 14 days, six nurses in each of three wards used ordinary soap, and then hexachlorophene soap for all ablutions, and finally ordinary soap again. At the end of each week viable counts for total organisms and for presumptive *Staph. aureus* were made on standard handwashings in Ringer's solution from each of the nurses.

Table II shows that the counts of total organisms and of presumptive staphylococci were reduced by about two-thirds during the period when hexachlorophene soap was being used. Whereas 86% of the samples from hands washed with ordinary soap yielded *Staph. aureus*, these organisms were isolated from only 46% of the samples from hands washed with hexachlorophene soap; if the Burns Unit, in which the risks of contamination are exceptional, was left out of the assessment, the use of hexachlorophene soap was associated with a reduction from 79% to 33% of samples yielding *Staph. aureus*.

TABLE II

EFFECT OF HEXACHLOROPHENE SOAP ON HAND FLORA OF NURSES

	All Colonies		Staph. aureus	
Soap used in Ward and Off Duty	*Mean Count per 0·1 ml. Washings*	*Samples*	*Mean Count per 1·0 ml. Washings*	*Samples*
Ordinary (1st fortnight)	504 ± 72	35	72·1 ± 13	36
Hexachlorophene (2nd fortnight)	186 ± 74	42	29·7 ± 8·5	42
Ordinary (3rd fortnight)	635 ± 74	27	127 ± 52	27

THE OPERATION SITE

Our chief aim was to find an alternative to alcoholic iodine which would be as effective in reducing the skin flora but free from the risks of sensitization and irritation. We tested some of the newer antiseptics, including chlorhexidine, Virac (an iodophor), and Penotrane (phenyl mercuric dinaphthyl methane sulphonate); hexachlorophene was not included, because its slow action makes it unsuitable for disinfection of the operation site. In subsidiary experiments we examined the elimination of *Staph. aureus* from hand carriers, and the effect of successive treatments with antiseptic.

In the first experiment we used a method similar to that of Story (1952) to assess the effect of antiseptics on staphylococcal cultures deposited and allowed to dry on the skin (Table III). Each of the antiseptics except aqueous Penotrane and cetrimide removed all detectable staphylococci from the surface of the skin during two minutes' exposure to the antiseptic.

In the second experiment (Table III) we examined the effect of antiseptics on the resident flora. Six antiseptics which had removed all detectable organisms in the first experiment were examined with controls by a standard handwashing technique on seven subjects. Viable counts were made of washings taken before and after two minutes' treatment with antiseptic or (in the controls) before and after a rinse in running water. A Latin square design was

TABLE III

COMPARISON OF EFFECTS OF DIFFERENT ANTISEPTICS ON SUPERFICIAL AND ON RESIDENT FLORA OF SKIN

Antiseptics	*Approximate Duration of Treatment (min.)*	*Superficial Organisms (culture of staphylococci) Applied to Skin*		*Resident Organisms*	
		Mean % Organisms Surviving after Treatment	*Tests*	*Mean % Organisms Surviving after Treatment*	*Tests**
None	—	—	—	85·3	7
Ethyl alcohol (70%)	0·4	0·008 – 1·2	4	—	—
	2·0	<0·0004	3	31·4	7
Iodine (1%) in ethyl alcohol (70%)	0·4	<0·012 – 0·24	7	—	—
Iodine (1%) in ethyl alcohol (70%)	2·0	—	—	19·3	7
Chlorhexidine (0·5%) in ethyl alcohol	0·4	0·002 – 0·03	2	—	—
Chlorhexidine (0·5%) in ethyl alcohol	2·0	—	—	18·7	7
Lugol's iodine	2·0	<0·0003	2	32·6	7
Virac	2·0	<0·0003	2	41·2	7
Chlorhexidine (0·5%) in water	2·0	<0·0003	2	39·3	7
Cetrimide (2%) in water	2·0	>0·09 – >0·14‡	2	—	—
Penotrane (0·1%) in water	2·0	6·4 – 12·2	4	(67·2)†	2

* Latin square design.
† Separate experiment, not included in Latin square design.
‡ Inhibition on culture plate not completely neutralized.

used for the experiment. Chlorhexidine (0·5%) in 70% ethyl alcohol was found to be as effective as 1% iodine in alcohol, and significantly more effective than any of the other methods tested; the best method reduced the skin flora by approximately 80%.

Nurses' hands were usually found to lose *Staph. aureus* in about the same proportions as they lost total organisms after disinfection with alcohol; in these subjects staphylococci were obviously carried with the resident rather than the transient flora. Attempts to improve the effectiveness of disinfection by prolonging the period of exposure to an antiseptic (ethyl alcohol) were disappointing; four minutes' treatment was sometimes more effective than two minutes' treatment, but a longer period of disinfection did not cause any further reduction in the bacterial flora, presumably because the remaining bacteria were too deeply placed to be reached by the antiseptic.

COMMENTS

The limited effect of antiseptics on the resident flora is disappointing. Some improvement can be expected by preliminary vigorous cleansing with detergents, *e.g.*, quarternaries, and fat solvents, and by the application of antiseptics with friction (Price, 1938); but the inaccessibility of the deeper flora to agents applied at the surface would seem to limit the possibilities of skin disinfection. This *impasse* might be overcome by the use of an antiseptic that penetrates to the deeper layers; a compound of this kind (Penotrane) was tested (see Goldberg, Shapero and Wilder, 1950), but in our study its effects were largely bacteriostatic. Fortunately *Staph. aureus* is usually absent or sparsely represented in the skin. Moreover, Elek and Conen (1957) have shown that a million or more virulent staphylococci are required for the initiation of a suppurating infection. If an antiseptic does nothing more than eliminate the transient flora and reduce the numbers of the resident skin staphylococci this may, nevertheless, prevent the development of sepsis by reducing the inoculum of staphylococci at the site of surgical incision or puncture.

Price (1938) has shown that the skin flora return gradually over a period of several days to the original level after effective disinfection. For this reason it should be possible to maintain the bacterial flora of the hands of nurse or surgeon at a low level without excessive rubbing when antiseptics such as hexachlorophene are used regularly. Apart from some drying of the skin, hexachlorophene appears to have little if any tendency to cause skin reactions. Sensitization to bacitracin also appears to be very rare (Jawetz, 1956) but skin reactions to neomycin are occasionally reported (Calnan and Sarkany, 1958).

Repeated disinfection on successive days is sometimes used for operation sites, especially in orthopaedic surgery, but it is doubtful if the level of colonization is lower after this procedure than it is after a single thorough disinfection on the operating table. Repeated cleansing with detergents is desirable before operations on skin containing ingrained dirt, and especially on the hands of gardeners and agricultural workers which may carry large numbers of clostridial spores; these cannot be destroyed by antiseptics or completely removed by

one cleansing with soap and water, but as they belong to the transient flora they are gradually shed from the skin with the horny layer.

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