

Polyvinyl-pyrrolidone-iodine: an assessment of antibacterial activity

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As a general disinfectant, iodine is one of the most powerful substances known. It has, however, well-known disadvantages such as irritation of the skin and mucous membranes, staining of the skin and fabrics, and a liability to cause iodism on continued use. In an attempt to overcome these disadvantages, Shelanski & Shelanski (1956) used a combination of iodine with polyvinyl pyrrolidone (PVP-I). This complex is known to possess considerable power of disinfection and shows promise as a skin disinfectant.

Disinfection of the skin as a preparatory measure in surgical procedures still presents many problems, especially in babies. This is particularly the case when surgery has to be practised in an area where even commensal organisms such as *Staphylococcus albus* can cause infection, e.g. in the repair of congenital deformities. The present investigation was designed to explore the possibility of using iodine in the form of the complex with polyvinyl pyrrolidone for this purpose.

METHODS

In these studies the following two PVP-I preparations were investigated for their activity against micro-organisms.

PVP-I aqueous solution (PVP-I sol.)

PVP-I	1.0% available iodine
Glycerol	1.0% (v/v)
Nonyl phenoxy polyoxyethylene ethanol*	0.25% (v/v)
Buffer, disodium phosphate/citric acid	

PVP-I surgical scrub (PVP-I scrub)

PVP-I	0.75% available iodine
Lauric acid diethanolamine condensate*	4.0% (w/v)
Ammonium alkyl phenoxy polyoxyethylene glycol sulphonate*	25.0% (v/v)
Sodium hydroxide and hydrochloric acid to adjust pH to 4.6	

Action of PVP-I on vegetative micro-organisms

Non-spore-forming bacteria

An inoculum of 0.3 ml. of a 24 hr. nutrient broth culture of each of the test organisms was added to 3 ml. of PVP-I sol. at 20° C., giving a final bacterial concentration of approximately 1×10^8 organisms/ml. Standard 4 mm. loop samples were taken at 15, 30 and 45 sec. and at 1, 2, 3, 4, 5 and 10 min. into 3 ml. of

* Surface active agents.

nutrient broth containing sodium thiosulphate (1%) to neutralize any free iodine present. At this concentration sodium thiosulphate did not affect the growth of the test organisms in broth controls. All transplants were incubated for 3 days at 37°, then subcultured on to nutrient agar and read after overnight incubation. Modifications of this technique were carried out to investigate the effect of the presence of biological fluids and other factors described in the text.

Spore-forming bacteria

Vegetative cells of spore-forming bacteria were obtained by centrifuging 72 hr. broth cultures of the organisms, removing the supernatant medium, then adding the same volume of fresh medium and incubating for 4 hr. at 37° C. All clostridia were grown in Robertson's cooked meat medium and aerobic bacilli in nutrient broth containing soil extract (1%). The organisms were then tested against PVP-I sol. as described for non-spore-forming bacteria above. All transplants of clostridia were made into Robertson's cooked meat medium containing 1% sodium thiosulphate and of bacilli into nutrient broth containing 1% sodium thiosulphate. These were incubated for 3 days at 37° C., subcultured on blood agar anaerobically for clostridia and nutrient agar aerobically for bacilli, then read after overnight incubation.

Fungal hyphae

The hyphae from centrifuged 48 hr. Czapek Dox broth cultures (3 ml.) were used as inocula. To the hyphae after the removal of the supernatant medium 3 ml. of PVP-I sol. was added. Standard loop samples were taken as above on to Czapek Dox agar containing 1% sodium thiosulphate; all transplants were incubated at 25° C. for 7 days.

Action of PVP-I on spores

Bacterial spores

Seventy-two hour broth cultures of the test-sporing bacteria were used after microscopic examination to ensure that spores were present. Inocula (0.3 ml.) of these cultures were added to 3 ml. PVP-I sol. at 20° C.; standard loop samples were taken at 5, 10, 20, 30, 60, 120, 150 and 180 min. and then at hourly intervals to 5 hr. with a final transplant after 17 hr. All the test bacteria were grown in media as for vegetative cells of spore formers and incubated as in this method. This test was repeated for comparison with the following solutions of standard antiseptics at dilutions of 1/1, 1/10, 1/100 and 1/1000: chlorhexidine, 0.5% (w/v) aqueous; Lugol's iodine; lysol, 1/20 aqueous; potassium permanganate, 10% (w/v).

Fungal spores

Spores from 7-day plates of the test fungi were suspended in 3 ml. of sterile water. The spore suspension (0.3 ml.) was added to 3 ml. PVP-I sol.; sampling, incubation and blanks were as for fungal hyphae.

Action of PVP-I on bacterial suspensions in fats and oils

The following lipids were used: glycerol triacetate, glycerol tributyrate, glycerol trilaurate, glycerol trioleate, glycerol tripalmitate, glycerol tristearate, glycerol monoricinoleate, arachis oil, coconut oil, olive oil. To 3 ml. of the sterile fat or oil was added 0.3 ml. of a 24 hr. broth culture of the test organism. The resulting mixture was well shaken for 5 min. and 0.3 ml. of the emulsion was added to 3 ml. of PVP-I sol.; sampling and incubation were as for non-sporing bacteria. As some emulsions tended to separate on addition to PVP-I sol., sampling was done carefully to ensure the transference of a fat droplet each time. The individual fats and oils were also allowed to remain in contact with each test organism without PVP-I sol. for 1 hr., and then subcultured into nutrient thiosulphate broth to exclude inhibition by the lipid. PVP-I sol. without the test fats or oils was also tested under similar conditions. This was repeated using PVP-I scrub and aqueous 0.5% chlorhexidine (w/v).

Sterilization of glass, plastic and metal surfaces

PVP-I was compared with the following standard disinfectants: acetic acid, 5% (v/v) aqueous; chlorhexidine, 0.5% (w/v) aqueous; chlorhexidine, 0.5% (w/v) alcoholic; dettol, 1/20 aqueous; Lugol's iodine; lysol, 1/20 aqueous; sterile distilled water.

Sterile glass and plastic Petri dishes were infected with 0.2 ml. of an overnight culture of the test organism and allowed to dry for 3 hr. The surfaces were then flooded with the test solutions; after 10 min. the solutions were tipped off and the surfaces rinsed gently in distilled water. The plates were then flooded with 6 ml. of nutrient broth and incubated for 24 hr. at 37° C. in a desiccator with a beaker of water to prevent drying. After incubation, the broths were subcultured on nutrient agar plates and incubated overnight, any growth being noted. This was repeated but the surfaces were covered with a thin layer of agar before contamination. Sterile aluminium foil (2.5 × 1.5 mm.), infected by dipping in a 24 hr. culture of the test organism, was then tested similarly. The foil was placed in 10 ml. nutrient broth and incubated. To determine the speed of action of PVP-I sol., infected foil was placed in the PVP-I (1/1 and 1/10) and foil samples withdrawn at 15, 30 and 45 sec. and 1, 2, 3, 4, 5 and 10 min.

Action of PVP-I on Trichomonas

Trichomonas vaginalis was washed from a swab into 5 ml. of sterile saline at 37° C. The resulting suspension was centrifuged; the sediment containing living *Trichomonas* was treated as follows:

- (1) A slide preparation in saline was kept as control.
- (2) A slide preparation in 1/20 PVP-I made up as a 'Douche' containing equal volumes of both preparations was examined microscopically, death being taken as the point when all movement of the organisms ceased.

Residual bactericidal activity of PVP-I after neutralization with sodium thiosulphate

The iodine in a 10 ml. sample of PVP-I sol. was neutralized by titration with sterile 1% sodium thiosulphate, the volume used was noted, and this volume of sterile distilled water was added to a further 10 ml. of PVP-I sol. A 3 ml. sample of each solution was taken and the remainder diluted 1/10 to 1/100,000 also in 3 ml. final volumes. To each solution and its dilutions was added 0.3 ml. of a 24 hr. broth culture of *Staphylococcus aureus*. Standard loop samples were taken at 3, 4, 5, 7, 9 and 10 min., transferred to 3 ml. nutrient thiosulphate broth, incubated at 37° for 24 hr., plated on to nutrient agar and re-incubated. A broth blank was set up as before and also one for the neutralized PVP-I sol. containing the same concentration of sodium thiosulphate.

Action of PVP-I on the skin flora

Unless stated otherwise, all samples of the skin flora in these studies were taken by a modification of the velvet pad replica-plate technique (Lederberg & Lederberg, 1952). The velvet pad was stuck on to aluminium foil to allow it to remain in apposition to the area being swabbed. Before use, the sterile pad was moistened in nutrient broth, then pressed over the area to be sampled; it was then pressed on to a horse blood agar which was incubated at 37° C. overnight; any colonies formed were counted, mapped and identified.

RESULTS

Action of PVP-I on vegetative micro-organisms

PVP-I sol. (1/1 and 1/10) killed the majority of vegetative test organisms within 30 sec. (Table 1), though some strains of *Staph. aureus* required 3 min. to be killed. The presence of 5% of serum or 10% of citrated whole blood in the antiseptic delayed the lethal action of PVP-I sol. on one strain of *Staph. aureus* by 15 sec. PVP-I sol. was tested at a dilution of 1/10 against 160 strains of vegetative bacteria, all isolated from clinical sources, using 0.3 ml. inocula containing approximately 10^{12} organisms/ml. All the test organisms were killed within 10 min., but at 5 min. 7 organisms (5 strains of *Staph. aureus*, 1 coliform and 1 *Pseudomonas pyocyanea*) remained alive. PVP-I sol. was however uniformly bactericidal at 1/100 dilution in 1 min. to lighter suspensions (0.3 ml.) of 10^9 organisms/ml. washed 24 hr. cultures (Table 1).

Action of PVP-I on spores

PVP-I sol. did not kill any of the bacterial spores tested within 17 hr. Lugol's iodine was the only test solution to show sporicidal activity, killing at all dilutions. Fungal spores were all killed within 90 min. by PVP-I sol. (Table 2).

Action of PVP-I on bacterial suspensions in fats and oils

The addition of tripalmitin, tristearin and, to a less extent, various other fats and oils caused a 3- to 20-fold delay in the bactericidal action of PVP-I, whether in aqueous solution or formulated as a 'scrub'.

The presence of the fats did not affect the growth of the test organisms after 1 hr. incubation. The interfering effect of these fats was stronger against PVP-I than against chlorhexidine (Table 3).

Table 1. Activity of PVP-I against vegetative micro-organisms

Test organisms (vegetative)	No. of strains tested		Control	PVP-I sol.			
	At all dilutions	At 1/10 only		1/1	1/10	1/100*	1/1000*
<i>Esch. coli</i>	2	9	G	K	K	K	G
<i>Proteus</i> sp.	2	10	G	K	K	K	G
<i>Ps. pyocyanea</i>	2	5	G	K	K	K	G
<i>Salm. typhi-murium</i>	1	2	G	K	K	K	G
<i>Shigella sonnei</i>	1	2	G	K	K	K	G
<i>Staph. albus</i>	2	27	G	K	K	K	G
<i>Staph. aureus</i>	4	85	G	K	K	K	G
<i>Staph. aureus</i>	1	6	G	K 3	K 3	K 3	G
Haem. Streptococcus							
Group A	1	6	G	K	K	K	G
Group B	1	1	G	K	K	K	G
Group C	1	1	G	K	K	K	G
Group D	1	1	G	K	K	K	G
<i>B. cereus</i>	1	—	G	K	K	—	—
<i>B. subtilis</i>	1	—	G	K	K	—	—
<i>Cl. septicum</i>	1	—	G	K	K	—	—
<i>Cl. sporogenes</i>	1	—	G	K	K	—	—
<i>Cl. tetani</i>	1	—	G	K	K	—	—
<i>Cl. welchii</i>	1	—	G	K	K	—	—
<i>Aspergillus flavus</i>	1	—	G	K	K	—	—
<i>A. niger</i>	1	—	G	K	K	—	—
<i>Candida albicans</i>	2	—	G	K	K	K	G
<i>Penicillium</i>	1	—	G	K	K	*	*

* Washed culture used as inoculum.

G, Normal growth as in controls; K, no growth after 30 sec.; K 3, no growth after 3 min.

Table 2. A comparison of antibacterial agents on bacterial and fungal spores

Test sporing organism	Chlorhexidine 0.5 % aqueous	Lugol's iodine	Lysol 1/20 aqueous	Potassium permanganate 10 %	PVP-I sol.	Control
Bacterial spores						
<i>Bacillus cereus</i>	G 17	K 180	G 17	G 17	G 17	G
<i>B. subtilis</i>	G 17	K 180	G 17	G 17	G 17	G
<i>Clostridium septicum</i>	G 17	K 180	G 17	G 17	G 17	G
<i>Cl. sporogenes</i>	G 17	K 180	G 17	G 17	G 17	G
<i>Cl. tetani</i>	G 17	K 180	G 17	G 17	G 17	G
<i>Cl. welchii</i>	G 17	K 180	G 17	G 17	G 17	G
Fungal spores						
<i>Aspergillus flavus</i>	—	—	—	—	K 15	G
<i>A. niger</i>	—	—	—	—	K 90	G
<i>Penicillium</i>	—	—	—	—	K 90	G

G, Normal growth as in controls; G 17, growth after 17 hr.; K 15, no growth after 15 min.; K 90, no growth after 90 min.; K 180, no growth after 180 min.

Sterilization of glass, plastic and metal surfaces

Of the 7 agents tested, all killed the test organism *Ps. pyocyanea* within 10 min. on clean surfaces. In the presence of agar, 1/20 Dettol was the only agent which failed to kill the test organism within 10 min. PVP-I sol. (1/1 and 1/10) killed the test organism on metal surfaces in 5 min.

Table 3. *Action of PVP-I on bacteria suspended in fats and oils*

Lipid vehicle for test organism	Mean time (min.) for 100 % bactericidal effect					
	Aqueous PVP-I		PVP-I scrub		Chlorhexidine aqueous 0.5 %	
	<i>Staph. aureus</i>	<i>Ps. pyocyanea</i>	<i>Staph. aureus</i>	<i>Ps. pyocyanea</i>	<i>Staph. aureus</i>	<i>Ps. pyocyanea</i>
Blank	3	< 0.5	3	< 0.5	< 0.5	< 0.5
Triacetin	2	< 0.5	3	< 0.5	< 0.5	< 0.5
Tributyrin	3	1	3	0.5	0.5	0.5
Trilaurin	5	2	5	2	—	—
Triolein	5	2	3	1	0.5	2
Tripalmitin	> 10	> 10	> 10	> 10	—	—
Tristearin	> 10	> 10	> 10	> 10	—	—
Monoricinoleate	3	2	3	2	> 10	> 10
Arachis oil	5	4	4	3	0.5	3
Coconut oil	> 10	3	3	2	2	1
Olive oil	> 10	2	4	2	2	1

Action of PVP-I on Trichomonas

All movements of the test organism ceased within 4 min. exposure to 1/20 PVP-I 'Douche'; this was taken as the killing time.

Residual bactericidal activity of PVP-I after neutralization with sodium thiosulphate

No residual bactericidal activity could be demonstrated in the neutralized PVP-I sol. against the test organism. The diluted PVP-I sol. (1/100) killed the inoculum within 3 min.

Action of PVP-I on the skin flora

PVP-I as a cream and scrub was compared with chlorhexidine (1 %, w/w, cream), diethyl ether, ethanol, fucidin (1 %, w/w, cream), soap (white).

The flora of the hand was sampled, and the hand completely covered with an agent and left for 5 min. without drying. The hand was rinsed and the flora sampled again. The results (Table 4) show that none of the skin preparations sterilized the skin surface completely. Diethyl ether produced an increased count, presumably by its solvent action on fats, releasing bacteria from the deeper layers of the skin. The experiment was then extended to include an ether wash after skin disinfection to see if the deeper layers of the skin were sterile (Table 4). This showed that many bacteria in the deeper layers of the skin were unaffected by any of the antiseptics used.

Action of PVP-I sol. on the flora of the hand

The flora of a normal, unwashed hand was sampled by the velvet-pad technique; dipped in PVP-I sol. for 5 sec. and shaken; after a further 25 sec. the hand was rinsed and the flora re-sampled. This was repeated with the same hand on subsequent days, using 1/5 and 1/10 dilutions of PVP-I sol. (Plate 1a). In the concentrated solution and at both these dilutions PVP-I sol. sterilized the surface of the hand by this method of sampling except in one instance in which one colony of *Staph. albus* remained. The average volume used for a pair of hands per dip was 6–12 ml.

Table 4. *Effect of surgical preparing agents on the skin flora*

Skin treated with	Mean colony count of sampled area* and standard error		Percentage reduction	Mean colony count of sampled area after ether†
	Before treatment	After treatment		
Chlorhexidine 1% cream (w/w)	82 ± 32	5 ± 1.44	94	25 ± 5
Diethyl ether	263 ± 113	—	—	581 ± 202
Ethanol	248 ± 145	5 ± 3.5	98	27 ± 22
Fucidin 1% cream (w/w)	237 ± 211	106 ± 74	55	289 ± 109
PVP-I cream	145 ± 123	3 ± 4	98	9 ± 2
PVP-I scrub	223 ± 260	0.66 ± 1.19	99	48 ± 45
Soap (white)	139 ± 14	55 ± 34	53	131 ± 33

* *B. subtilis*, *Staph. albus* and *aureus*, and *Sarcina*.

† *Staph. albus*.

In surgical gloves *Duration of action of PVP-I*

After the flora of the hand was sampled, the hand was scrubbed with PVP-I scrub for 5 min., rinsed, re-scrubbed for a further 5 min. and rinsed again. The skin was resampled and the hand placed in a surgical glove for 1 hr. After this period the glove was removed and the skin flora sampled. PVP-I scrub rendered the hand sterile on the surface; after 1 hr. in a surgical glove it remained sterile, as sampled by the velvet pad.

On exposed skin

A velvet pad swab of the hand was taken, the hand was then dipped in PVP-I sol. for 5 sec. After 1 min. the skin surface was re-swabbed and again at 30, 60, 90 and 120 min., the hand being used normally during this time. PVP-I sol. completely sterilized the skin surface and after 1 hr. it was still sterile; recolonization started after 1 hr.

The finger tips were deliberately infected with broth cultures of *Staph. albus*, *Esch. coli* and *Aerobacter aerogenes*, and then dabbed on a blood agar plate. The hand was then scrubbed in PVP-I scrub for two intervals of 5 min., rinsed and dried. The finger tips were then dabbed on to a blood agar plate, and again after 1 hr. This was repeated with chlorhexidine (0.5% in 1% stergene). The infected

finger tips were rendered sterile by PVP-I scrub, but with chlorhexidine in some cases some bacterial colonies remained. Recolonization of the finger tips started after 1 hr. (Plate 1*b*) with PVP-I scrub.

Bactericidal properties of residual PVP-I on the skin

The residual bactericidal properties of skin treated with PVP-I sol. were investigated as follows: The right hand was momentarily immersed in Lugol's iodine solution and the left hand in PVP-I sol. The hands were withdrawn and allowed to dry in air. After 1 hr., the right hand was placed into a beaker of 500 ml. of sterile water containing 1×10^7 *Staph. albus* per ml.; this was repeated with the left hand in another beaker. The hands were then agitated for 30 sec. to release any remaining antiseptic. After 2 min. viable counts were performed on the contents of the beakers. The hand previously dipped into PVP-I sol. killed the bacterial suspension in the beaker; the hand dipped into Lugol's iodine did not, a count of 5×10^6 organisms per ml. being recorded. A modification of the above experiment to determine if the skin surface became infected when immersed in bacterial suspensions was performed as follows: The flora of the finger tips was sampled before and after dipping them into PVP-I sol.; the residual PVP-I sol. was left on the skin for 1 hr. and the finger tips then dipped into suspensions of *Staph. albus*, *Esch. coli* and *A. aerogenes* (1×10^7 organisms per ml. in 15 ml. of distilled water); the finger tips were agitated for 30 sec., removed, and after 1 min. the skin surface was sampled; the bacterial suspensions were also sampled with a standard loop on nutrient agar, incubated for 12 hr. at 37° C.

Table 5. *A comparison of PVP-I with chlorhexidine-ether-meth. in skin preparation for ventricular tap and surgery*

Patient	Mean colony count (PVP-I preparation)		Patient	Mean colony count (Chlorhexidine-ether-meth. preparation)	
	Before	After		Before	After
1	41*	0	A	29*	3*
2	134*	0	B	36*	4*
3	10†	0	C	8*	0
4	51*	4*	D	39*	7*
5	196*	0	E	11*	0
6	32*	0	F	154*	11*

* *Staph. albus*.

† Gram-positive rods.

One minute after dipping into the bacterial suspensions, the finger tips were sterile. Samples from all three bacterial suspensions were sterile except in one isolated instance in which four colonies of *A. aerogenes* were re-isolated.

Effect of PVP-I on the skin of the head

The heads of 6 children were shaved before the performance of intraventricular and other neurosurgical procedures. The skin was sampled. PVP-I scrub was then used, followed by a rinse, application of PVP-I sol. and re-sampling of the skin.

The results (Table 5) show that the treatment with PVP-I was more efficient than that with chlorhexidine. Repeated applications of PVP-I produced no sensitizing or other reactions.

In the course of these experiments with PVP-I, 2 subjects exhibited mild iodism, each being hypersensitive to other forms of iodine.

DISCUSSION

These results show that PVP-I has a rapid lethal action on vegetative cells of various bacteria and fungi *in vitro*. Many organisms, including bacteria resistant to various antibiotics, are killed in less than 1 min. When dense suspensions are used, certain organisms may survive for 5 min. but not longer. This rapid lethal effect is due to the liberation of free iodine from the complex, and is antagonized completely by the addition of sodium thiosulphate. Smooth surfaces such as glass and metal are also rapidly disinfected. The presence of organic material, such as agar, serum or whole blood, serves to delay but not to abolish the rapid bactericidal effect.

The action of PVP-I on spores is much weaker. Fungal spores are killed in $1\frac{1}{2}$ hr. but spores of clostridia and other bacilli survive for 17 hr. This is in contradiction to the report by Gershenfeld (1962) that PVP-I kills spores of *Bacillus subtilis*, *Clostridium tetani* and *Cl. welchii* in $2\frac{1}{2}$ hr.

In its action on the skin flora, PVP-I appears to be more effective than several other agents commonly used in preparing the skin for surgical procedures, in that it renders the skin surfaces sterile. This agrees with the findings of Lenhardt & Lachapelle (1961) and Joress (1962). Lowbury, Lilly & Bull (1963) found that PVP-I scrub was comparable with 2% hexachlorophene soap. None of the agents used sterilizes the deeper layers of the skin, but PVP-I maintains a sterile skin surface for 1 hr. even in surgical gloves. Re-infection of a hand within 1 hr. after treatment with PVP-I is virtually impossible because of the residual PVP-I on the skin surface, which is enough to kill heavy bacterial suspensions in water. For general ward use PVP-I is acceptable. The prepared area is clearly defined by its colour but, unlike other formulations of iodine, it is readily washed out of skin and fabric by water. In our series, mild iodism occurred in two hypersensitive subjects. This is contrary to the assertion of Shelanski & Shelanski (1956) that PVP-I does not evoke this reaction.

SUMMARY

Polyvinyl-pyrrolidone-iodine complex (PVP-I) was shown to have a rapid disinfectant activity *in vitro* against vegetative bacteria and fungi and against vegetative *Trichomonas*. The presence of organic matter had a slight delaying effect on the killing time. Bacterial spores survived in PVP-I for 17 hr. and fungal spores for $1\frac{1}{2}$ hr. Used as an agent for pre-operative preparation of the skin, PVP-I was more efficient than others tested, rendering the skin surface sterile even when heavily contaminated, and keeping it so for 1 hr. None of the staining characteristics of iodine were noted since PVP-I was easily removed by water. Two individuals, known to be iodine sensitive, developed mild iodism after application of PVP-I.

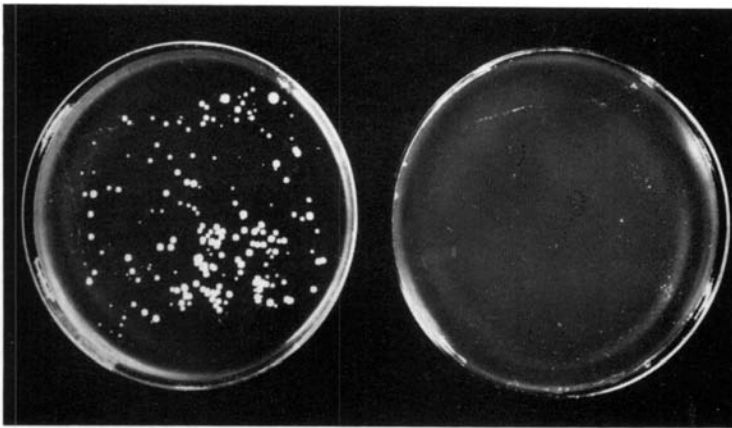
The authors are indebted to the staff of the hospital for their co-operation; and to Berk Pharmaceuticals Limited for supplies of PVP-I, manufactured by them as Betadine (Povidone-iodine B.P.).

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EXPLANATION OF PLATE 1

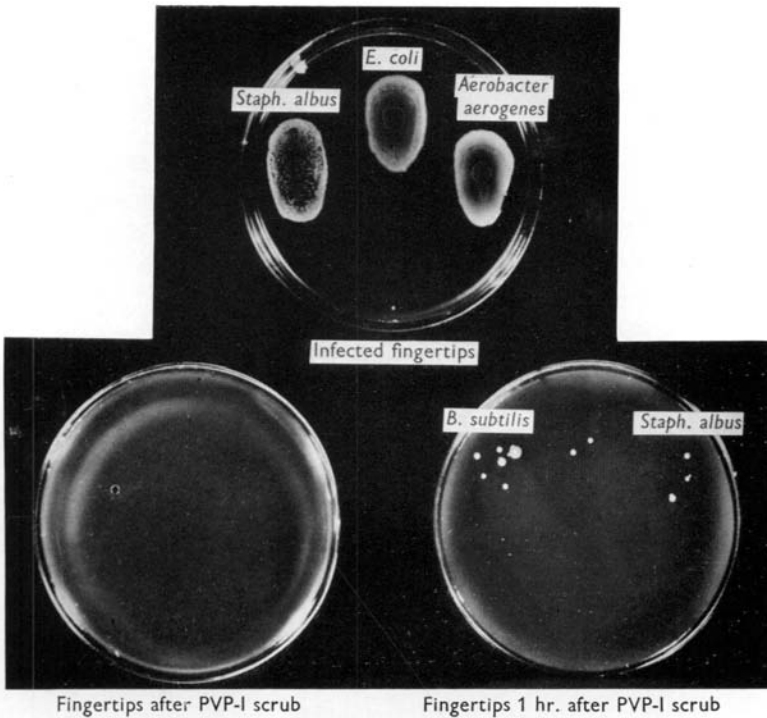
- (a) Velvet pad samples of normal skin surface flora (*Staph. albus*) and skin surface after a 5 sec. dip in PVP-I sol.
- (b) Finger-print plates, show the sterilization of heavily infected finger tips by PVP-I scrub and re-colonization of the skin surface after 1 hr.



Normal skin surface flora

Skin surface after PVP-I sol.

(a)



(b)