Papers and Originals

Sources of Gas Gangrene in Hospital

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Summary : Of four cases of postoperative gas gangrene in three hospitals three followed amputation of legs with gangrenous lesions, and one followed gastrectomy. Clostridium welchii was isolated from the wounds and the faeces of each patient ; small numbers of Cl. welchii were found on the floors of the theatres where the operations had been performed.

Two infections occurred in one hospital on successive days. Typing of strains of Cl. welchii from these patients showed that they were serologically distinct. Further studies suggested that in each of the four cases infection was probably acquired from the patients' intestinal flora, probably through faecal contamination of skin.

In 76 patients sampling of the skin with surface contact plates showed occasional heavy contamination of the thighs, groins, and buttocks with Cl. welchii, most of which were present as spores or sporing bacilli ; Cl. welchii was more commonly found in patients with incontinence of faeces. Compresses of povidone-iodine applied for 30 minutes were found greatly to reduce the numbers of Cl. welchii, and swabbing with 70% alcohol was effective in some cases ; washing with soap and water had no effect on the numbers of Cl. welchii on the skin.

Introduction

Mid-thigh amputation in patients with obliterative arterial disease is known to carry a risk of gas gangrene (Taylor, 1960). This was illustrated by Parker (1967), who reported 20 cases of postoperative sepsis due to *Clostridium welchii* occurring in 17 different hospitals over a period of nine months. Nearly all of the operations were on the lower limb and involved bone ; arterial insufficiency was clearly an important factor. Contamination with *Cl. welchii* from the patients' faeces seemed the most likely source of these infections, but contamination from the environment could not be ruled out, as the organism is commonly present in the dust and air of hospital wards and operating-suites (Lowbury and Lilly, 1958). Hospital infection with *Cl. welchii* probably acquired from the environment has been reported (Sevitt, 1953), and it is commonly assumed to occur.

This paper includes a brief account of three episodes of postoperative gas gangrene in which agglutination tests on *Cl. welchii* provided evidence of the likely sources of the strains causing infection. Studies were also made on the frequency and degree of contamination with *Cl. welchii* of the skin of hospital patients, on their presence in these areas as spores, and on methods of skin cleansing and disinfection to remove *Cl. welchii* before operations.

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Bacteriological Methods

Culture Medium

A selective medium for *Cl. welchii* containing neomycin (100 μg./ml.) and a substrate for the detection of lecithinase (Lowbury and Lilly, 1955) was used ; this medium (N.N.A.) was prepared either with 20% human serum, as in the original description, or with egg yolk as the source of lecithin (see Cruickshank, 1965) ; colonies growing on this medium and showing haloes of turbidity after 18 hours' incubation in an anaerobic jar were described as presumptive *Cl. welchii*. Few colonies of other species and no lecithinase-producing colonies of other species have been found to grow on this medium ; nevertheless, a proportion of colonies from samples showing presumptive *Cl. welchii* were given confirmatory tests by inoculation across an N.N.A. plate one-half of which was spread with *Cl. welchii* antitoxin to neutralize the specific lecithinase of *Cl. welchii* (Lowbury and Lilly, 1955).

Sampling Methods

Material from wounds and faeces was examined by the pathologists in the hospitals where the patients were treated, and strains of *Cl. welchii* isolated from these sources were sent to us for further study.

Swabs from floors and other surfaces were inoculated on N.N.A. medium and into cooked meat broth ; the latter was subcultured, after 24 hours' incubation at 37° C., by spot-inoculation on plates of N.N.A. medium. Cultures on N.N.A. medium were examined after overnight incubation in an anaerobic jar. Counts of *Cl. welchii* in the air were obtained with a large slit-sampler on plates of N.N.A. medium ; samples of 150–250 cu. ft. (4·2–5·6 cu. m.) of air were examined.

The skin and some inanimate surfaces were sampled for *Cl. welchii* by the use of surface contact plates containing N.N.A. medium. Alne disposable surface contact plates (a modification of those described by Hall and Hartnett (1964)) were filled with medium so that the surface was slightly above the rim of the plate. Areas of skin were sampled by pressing the contact plates briefly but firmly against the skin. After overnight incubation in an anaerobic jar the plates were examined for presumptive *Cl. welchii* ; the numbers of colonies on plates were counted.

Infections with Clostridium welchii

Three episodes of postoperative gas gangrene in different hospitals were studied, in collaboration with the medical staffs of the hospitals where they occurred. Strains of *Cl. welchii* were obtained from the wounds and faeces of the infected patients and from floor dust in the patients' environment. Anti-

sera prepared against strains of *Cl. welchii* were used to provide evidence of identity or difference between the strains isolated from these sources.

Preparation of Antisera and Agglutination Tests

In the first episode strains were examined at the Central Public Health Laboratory, Colindale, with a set of antisera prepared against food-poisoning strains of *Cl. welchii*.

Strains isolated from wounds and faeces in the second and third episodes were used for preparation of vaccines, and rabbits were immunized with these vaccines as described by Hobbs *et al.* (1953). Smooth colonies were picked from blood agar cultures and transferred to 1% glucose broth; after 18 hours' anaerobic incubation cultures were tested for purity, washed twice with distilled water, and resuspended in 0·1% formol saline. The density of the suspension was adjusted to $1{\cdot}5 \times 10^9$ organisms per ml. on Brown's turbidity scale. After two days the suspensions were tested for sterility. Rabbits were given successive doses, by intravenous injection every two to three days, of 0·1, 0·2, 0·4, 0·6, 0·8, and 1 ml. of the vaccine, each injection being made up to 1 ml. with physiological saline.

Rabbits were bled 10 days after the last injection. Strains of *Cl. welchii* were tested by slide agglutination with serum diluted 1/5; if agglutination occurred, tube agglutination tests were made in 3 by ½ in. (7·5 by 1·3 cm.) tubes with sera diluted serially from 1/5 to 1/1,280 and suspensions at 500×10^8 ml. on Brown's turbidity scale. Tubes were examined after incubation at 43° C. for four hours and again after two hours' further incubation at room temperature and overnight incubation at 4° C.

First Episode. Infections

In a general hospital two patients had operations on successive days. In both patients gas gangrene was diagnosed and ***Cl. welchii*** was isolated from the operation wound and from the faeces. The patients were in different wards and had their operations performed by different surgeons in different operating-theatres: it seemed improbable, however, that two infections of such rarity should occur in the same hospital, at the same time, unless an extraneous source was responsible. The episode was therefore investigated as a probable outbreak. Of the two patients, one (A. B.) was a woman aged 81 years with a gangrenous toe, whose leg was amputated below the knee. On the next day a clinical diagnosis of gas gangrene was made, and abundant *Cl. welchii* was obtained in cultures from the wound; numerous Gram-positive rods were found in the exudate and in sections of dead muscle. The faeces yielded *Cl. welchii* (10×10^6/g.) which resisted heating at 100° C. for one hour.

The other patient (C. D.) was a male, whose leg was amputated above the knee because of an intractable ulcer. Signs of gas gangrene appeared on the day after operation, and the patient died on the second day. *Cl. welchii* (heat-resistant) was isolated from the amputation stump, the faeces, and the blood.

Sampling of air and surfaces in the theatres where operations on these patients had been performed showed counts of *Cl. welchii* within normal limits (0·7 colony of *Cl. welchii* per 100 cu. ft. (0·25 colony per sq. m.) in the air and 2·7 colonies per 100 sq. cm. (1 colony per sq. m.) on the theatre floor); no *Cl. welchii* were isolated from other surfaces or equipment. The theatres were of modern design and plenum ventilated. The system of cleaning and sterilizing of instruments, bowls, dressings, and other materials was efficient, and satisfactory results were obtained in daily Bowie-Dick tests of the pressure steam sterilizers. All amputation instruments had been sterilized twice between cases. Cultures taken from amputation instruments showed no *Cl. welchii*. Cultures of water from water sterilizers, of bottled saline, and of sterilized glove powder showed no bacterial growth.

First Episode. Serological Tests on Cl. welchii

Agglutination tests reported by Dr. M. T. Parker using antisera to food-poisoning strains showed the strain of *Cl. welchii* from both wound and faeces of patient C. D. to be of serotype 10, while the strain from the other patient and one strain from the environment were untypable with the sera available. The strains causing the two infections with *Cl. welchii* were therefore shown to be different from each other, and in one case there was presumptive evidence of self-infection, the identical serotype of *Cl. welchii* being isolated from the faeces and the wound of the same patient.

Second Episode. Infection

In another general hospital a patient with a gastric ulcer and haematemesis developed jaundice and fever 24 hours after a difficult gastrectomy. Signs of gas gangrene were noted. On removal of sutures, pus with bubbles of gas and necrotic muscle were seen. Gram-positive bacilli were seen in microscopic films of the pus, on culture *Cl. welchii* was isolated from the wound and also from the patient's faeces. The patient died, and *Cl. welchii* was isolated from the wound, the faeces, and jejunal contents.

A survey of the environment showed airborne *Cl. welchii* within normal limits. *Cl. welchii* was found in one sample from the operating-theatre floor. A number of samples from floors and other surfaces in the operating-theatres did not yield *Cl. welchii*.

Second Episode. Serological Tests on Strains

Antisera prepared against the strains of *Cl. welchii* from the wound and from the faeces of the patient gave rapid slide agglutination and tube agglutination to a titre of 1 in 80 with both wound and faecal strains of *Cl. welchii*, but not with strains isolated from the patient's jejunal contents, from the operating-theatre floor, or from the patient infected during the third episode (Table I).

TABLE I.—*Agglutination of Cl. welchii from Wounds, Faeces, and Environment by Antisera*

Strain of *Cl. welchii*	Source of Strain	Agglutination by Antisera to Strains*			
		763	764	3726	3727
3726	Episode 2, wound	0	0	80	80
3727	Episode 2, faeces	0	0	80	80
3725	Episode 2, theatre floor	0	0	0	0
3728	Episode 2, jejunal contents	0	0	0	0
763	Episode 3, wound	40	80	0	0
764	Episode 3, faeces	40	80	0	0
774	Episode 3, theatre floor	0	0	0	0
II	Episode 3, theatre floor	0	0	0	0

* Numbers indicate titres in agglutination tests. 0 indicates no slide agglutination.

Third Episode. Infection

A patient with diabetic gangrene, who had had a mid-thigh amputation of the leg, developed signs of gas gangrene four days after the operation; the infection spread to the abdominal wall. Large doses (4 mega units) of penicillin and 500 mg. of tetracycline were given four-hourly, and the patient was transferred to another hospital for treatment with hyperbaric oxygen. The patient made a good recovery and was discharged from the hospital where hyperbaric oxygen treatment was given.

Cl. welchii was isolated from the patient's wound and faeces and from the floor of the operating-theatre where the patient's leg had been amputated.

Third Episode. Serological Tests on Strains

The antiserum prepared against the strain of *Cl. welchii* isolated from the patient's wound agglutinated the immunizing strain and the strain isolated from the patient's faeces; antiserum prepared against the strain isolated from the patient's faeces agglutinated the strains from the wound and from the faeces. Neither serum agglutinated the strains isolated in the second episode or the strain isolated from the floor of the operating-theatre where the patient's limb had been amputated (see Table I).

The findings in these three episodes supported the view that in each case self-infection had occurred with *Cl. welchii* from the patient's faeces. Prophylactic antibiotic therapy had not been used in the patients who developed gas gangrene.

Contamination of Skin with Cl. welchii

Cl. welchii is found, often in large numbers, in the faeces of most healthy persons. It seems probable that self-infection with *Cl. welchii* after operations on the leg is due to contamination with clostridial spores of faecal origin which are present on the skin of the operation site.

To obtain information on this hazard we examined the skin of 76 patients from *Cl. welchii* in the wards of two hospitals: we also examined strains isolated from the skin for the presence of spores and made a few tests on the possible value of alternative methods for removal of the clostridia in preoperative cleansing and disinfection of the skin.

Surveys of Cl. welchii on Skin

Twelve patients (seven female and five male) in a geriatric hospital (hospital A) and 57 patients (20 female and 37 male) at the Birmingham Accident Hospital (hospital B) were sampled for *Cl. welchii* with surface contact plates as described above; samples were taken from a standard range of sites on buttocks, groins, and thighs. In a smaller series (seven male patients) samples were taken from a wider range of skin sites. Some of the patients were known to have incontinence of faeces. One patient with a colostomy was sampled.

Results

Table II shows the numbers of *Cl. welchii* isolated from the skin of the buttocks, groins, and right and left thighs of the 57 patients sampled in hospital B. None of these patients was incontinent of faeces. No *Cl. welchii* were isolated from 145 of the 207 (70%) sites, and between 1 and 10 colonies were isolated from 48 (23%) sites. A heavy growth of *Cl. welchii* was isolated from most of the sites examined on two elderly female patients (see Fig. 1). More than 10 colonies on at least one site were isolated from six patients, five of whom were female. The results of sampling similar sites in 12 patients with faecal incontinence in hospital A are shown in Table III. In these patients *Cl. welchii* was not isolated from 14 of the 48 (29%) sites and between 1 and 10 colonies were isolated from 11 (23%) sites. Five of the 12 patients showed a heavy growth on at least one site, and nine showed 10 or more colonies; seven of these patients were female and two were male. Table IV shows the results of sampling a larger number of sites on five geriatric patients with faecal incontinence and on two geriatric patients without incontinence. One patient showed heavy contamination on most of the body sites examined. In the other patients, both incontinent and continent, *Cl. welchii* were isolated in small numbers. Isolation of *Cl. welchii* from the buttocks and perineum was surprisingly infrequent, whereas the palm of the hand was usually contaminated.

A patient with a colostomy was sampled for *Cl. welchii* after passage of faeces and cleansing of the skin with soap and water. Contact plate samples were taken from four skin sites around

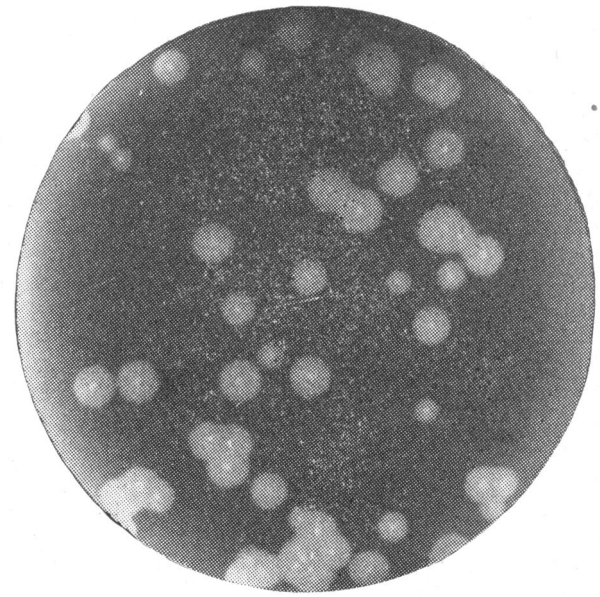

FIG. 1.—Surface contact plate culture on N.N.A. medium, showing heavy growth of *Cl. welchii* on sample taken from the skin of buttocks.

TABLE II.—*Isolation of Cl. welchii From Skin of Hospital Patients*

Sites	Total Sites	Colonies of *Cl. welchii* per Plate					
		0	1–3	4–10	11–50	51–100	>100
Buttock ..	47	34	6	4	2	0	1
Groin	57	38	11	2	4	0	2
Right thigh ..	50	38	8	1	1	1	1
Left thigh ..	53	35	14	2	1	1	0
Total	207	145	39	9	8	2	4

TABLE III.—*Isolation of Cl. welchii From Skin of Incontinent Geriatric Patients*

Site of Sampling	Total Sites	Colonies of *Cl. welchii* per Plate					
		0	1–3	4–10	11–50	51–100	>100
Buttock ..	12	2	2	1	2	0	5
Groin	12	3	2	1	3	0	3
Left thigh ..	12	5	1	1	3	1	1
Right thigh ..	12	4	3	0	3	1	1
	48	14	8	3	11	2	10

TABLE IV.—*Isolation of Cl. welchii From Various Skin Sites on Five Male Patients with Faecal Incontinence and Two Normal Geriatric Patients*

Site of Sampling	Colonies of *Cl. welchii* per Plate						
	Incontinent Patients					Non-incontinent Patients	
Hair	56	1	0	0	0	1	1
Nose	0	0	0	1	0	0	0
Back	—	2	1	—	0	0	0
Chest	14	0	0	—	—	—	—
Abdomen	>100	0	0	0	0	1	0
Groin	>100	0	0	4	0	0	2
Palm of hand	>100	1	—	8	4	3	2
Buttock	SC	1	0	0	1	0	0
Back of thighs { Upper	SC	6	3	5	0	0	1
Back of thighs { Lower	—	1	0	2	0	0	0
Perineum	SC	0	0	—	0	0	0
Sole of foot	54	1	2	0	0	1	0

SC = Semiconfluent. — Not tested.

the colostomy and from both thighs. More than 300 colonies of presumptive *Cl. welchii* were obtained from one sample taken near the colostomy; the other three sites near the colostomy yielded 21, 5, and 0 colonies, and no colonies of *Cl. welchii* were isolated from the thighs.

Presence of spores of Cl. welchii on Skin

The removal of clostridia from the skin presents a special problem, because methods of disinfection which are effective against vegetative bacteria are inadequate against spores. To determine whether *Cl. welchii* on the skin are present as sporing organisms, a swab moistened with peptone water was rubbed over a skin site which had shown large numbers of *Cl. welchii* in a previous sampling. The swab was then rubbed off under the surface of a tube containing 1 ml. of Ringer's solution. Half of the contents were transferred to another tube. One tube was heated at 80° C. for 10 minutes; this treatment had been shown to destroy non-sporing forms of *Cl. welchii*.

Surface viable counts were obtained by spreading 0·2-ml. amounts of both heated and unheated suspension over the surface of N.N.A. plates and examining the plates after overnight anaerobic incubation.

Results

Small numbers of presumptive *Cl. welchii* were found on plates inoculated with heated samplings (six colonies) and with unheated skin samplings (10 colonies); a large proportion of the organisms were therefore inferred to be present as sporing clostridia.

Removal of Cl. welchii from Skin

In two patients skin sites on which large numbers of *Cl. welchii* had been found in previous tests were examined as follows. The contaminated area was divided into six smaller areas. From all six areas surface contact samples were taken on N.N.A. medium. Two of the smaller areas were then treated by application of compresses of povidone antiseptic solution, which were left in place for 30 minutes; another two of these areas were washed with soap and water and dried with a towel; and the two remaining small areas were left untreated as controls. A second surface contact plate sample was then taken from each of the areas on N.N.A. medium.

Four, including one of the above patients, were also tested for removal of the clostridia by rubbing the contaminated area with swabs moistened with 70% ethyl alcohol. Two tests were made on three of these patients. The areas were sampled by surface contact plates of N.N.A. medium before cleansing and again immediately after the cleansed skin appeared dry.

Results

The results are shown in Table V. Treatment with the local application of povidone-iodine compresses was associated with a considerable reduction in counts of *Cl. welchii* in Cases 1 and 2 (see Figs. 2 and 3).

Cleaning with 70% alcohol was quick; in Cases 1 and 4 it was effective, but not in Case 5. Washing with soap and water was ineffective. Small numbers of colonies were isolated in Case 3, both before and after cleaning with 70% alcohol. It is difficult to draw conclusions from these results, because of the irregular distribution of *Cl. welchii* on the skin and the day-to-day variations in counts from the same area, especially in incontinent subjects. It is obvious that none of the methods was entirely successful or reliable.

TABLE V.—*Effect of Treatment on Number of Cl. welchii Isolated from Skin of Patients*

Case No.	Time of Sampling	Colonies of *Cl. welchii* per Plate				
		Control (No Treatment)	Washing with Soap and Water	Povidone-Iodine Compresses	Swabbing with 70% Alcohol	
					Expt. 1	Expt. 2
1	Before treatment	60	84	> 1,000	46	19
	After treatment	725	52	36	3	0
2	Before treatment	41	20	91	—	—
	After treatment	38	22	9	—	—
3	Before treatment	7	—	—	2	6
	After treatment	6	—	—	6	2
4	Before treatment	11	—	—	50	—
	After treatment	80	—	—	1	—
5	Before treatment	> 1,000	—	—	> 1,000	170
	After treatment	750	—	—	960	181

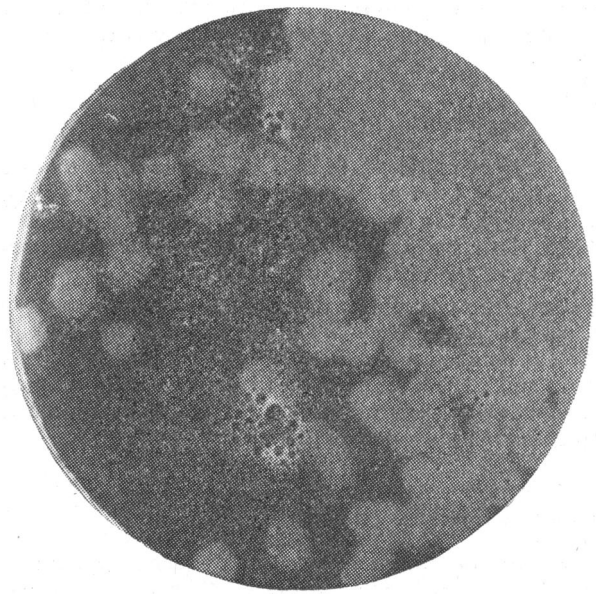

FIG. 2

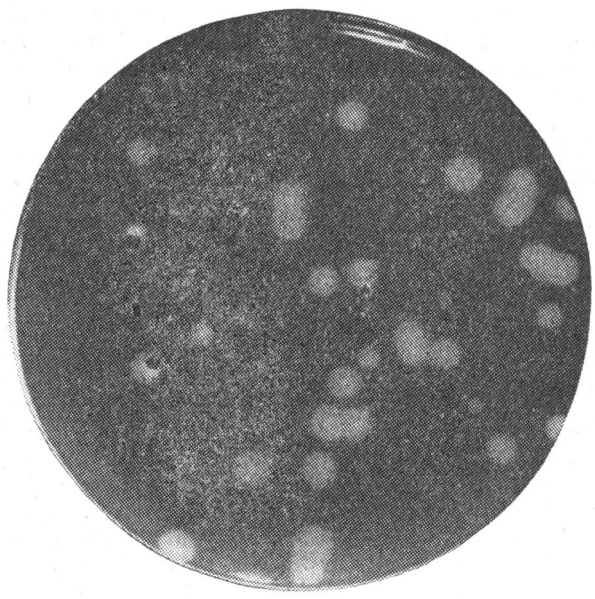

FIG. 3

FIGS. 2 and 3.—Surface contact samples on N.N.A. medium taken from buttocks before and after treatment with povidone-iodine compresses (see Case 1, Table V). The second sample shows much smaller numbers of *Cl. welchii*, but the clostridia were irregularly distributed in the first sample.

Discussion

The first question considered in this paper was whether four patients with postoperative gas gangrene in three hospitals acquired the infection from the environment or from their own intestinal flora. Agglutination tests with antisera prepared against *Cl. welchii* showed that the strains isolated from the wound and from the faeces of individual patients were indistinguishable from each other but different from those isolated from the patient's environment and from strains isolated from other patients. As strains of *Cl. welchii* can be shown by agglutination tests to be very heterogeneous (Henderson, 1940), the cross-agglutination of strains from faeces and wounds by antisera prepared against both strains was, in each case, strong presumptive evidence of self-infection. The likelihood that self-infection is a common source of *Cl. welchii* in burns was shown some years ago by the greater incidence of such infection when *Cl. welchii* has been isolated from rectal swabs taken on admission than when rectal swabs did not yield the organism (Lowbury and Lilly, 1958).

In addition to the hazards of self-infection at operation on patients with poor circulation (Taylor, 1960; Parker, 1967), there is a recognized hazard of gas gangrene on intramuscular injection of adrenaline (Cooper, 1946; Marshall and Sims, 1960). These are probably due to self-infection with faecal organisms on the skin, and for this reason it has been recommended that adrenaline should not be injected into the buttock (Harvey and Purnell, 1968).

If the patient's faecal flora are the most important source of postoperative wound contamination with *Cl. welchii* leading to gas gangrene, they are most likely to gain access from the skin contaminated with faeces. A small proportion of patients in hospital were found to have moderate or large numbers of *Cl. welchii* on the buttocks, groins, and thighs; skin sites remote from these areas were rarely heavily contaminated. Though patients with incontinence of faeces or with colostomies usually showed more skin contamination than others, heavy contamination was found in some other people. More female patients (12 out of 37) showed moderate or heavy contamination than did male patients (4 out of 39), whether incontinence of faeces was present or absent. The state of the skin, the numbers of *Cl. welchii* in the faeces, the amount of time spent in bed, and the necessity for bed-bathing are probably relevant factors but were not investigated. None of the younger people examined showed heavy skin contamination. Tests of heat resistance on clostridia removed from contaminated skin showed that a large proportion were present as spores or sporing bacilli.

The usual methods of skin disinfection are presumed to be inadequate for the removal of spores which are resistant or relatively insensitive to bactericidal compounds, but compresses of the iodophor povidone-iodine applied for 15 to 30 minutes have been found to have a useful effect (Lowbury *et al.*, 1964). Moreover, the clostridia are assumed to be "transient" rather than "resident" organisms and therefore probably removable to a large degree by ablution with soap and water. Tests on patients whose skin showed the presence of large numbers of *Cl. welchii* gave some support to the view that povidone-iodine compresses have a useful effect against these skin contaminants, but the effect was apparently smaller than that found in the studies with sporing cultures applied to the skin (Lowbury *et al.*, 1964). Washing with soap and water, which was expected to have some value, appeared to be without effect; this may have been due, in part, to the recontamination of the skin site with spores that had been separated by the ablution; in the previous studies reported (Lowbury *et al.*, 1964) running water was used to wash away the organisms dislodged by ablution.

An interesting observation was the apparently successful removal of *Cl. welchii* from the skin of two out of four patients by swabbing with 70% alcohol. The mechanism that achieved this result must have been the physical removal of superficial organisms, for alcohol is inactive against spores. This method alone was unsuccessful in one patient and a single treatment cannot be considered reliable. To achieve maximum effectiveness there is a case for applying a compress of povidone-iodine for 30 minutes before the operation, followed by disinfection of the operation site in the standard way, with an alcoholic solution of chlorhexidine (0·5%) or iodine (1%). If this double treatment by complementary methods is impractical, good physical removal of spores might be obtained by repeated cleaning with 70% alcohol.

Contamination of the environment of the operating-theatres with *Cl. welchii* was generally low. Counts of airborne *Cl. welchii* in 10 operating-suites of other hospitals ranged between 0 and 3·6 organisms per 100 cu. ft. (0 and 1·3 organisms per sq. m.) (Ayliffe *et al.*, 1969). Floor contamination of theatre floors with *Cl. welchii* was generally low when compared with other areas in the hospital, especially if there was a well-defined clean zone in the theatre suite. The small amount of environmental contamination in most operating-theatres and the heavy contamination of the skin of some patients further suggest that self-infection is nearly always the mechanism responsible for gas gangrene infections in hospital.

The findings reported in this paper support the view that systemic treatment with a penicillin should be used as a prophylaxis against gas gangrene in patients who are at risk because of impaired arterial supply to operation sites, or who have reduced resistance to infection for any reason—for example, diabetes—and especially if there are known sites of heavy contamination. Such contamination can easily be detected by surface contact plates of a selective diagnostic medium; its presence can apparently be reduced, though not eliminated, by application of compresses of povidone-iodine, or less certainly by thorough cleaning with 70% alcohol.

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