Notes on the diagnosis and treatment of gas gangrene : with a suggested scheme for the bacteriological investigation of war wounds / War Wounds Committee and Committee of London Sector Pathologists.

M.R.C. War Memorandum No. 2

Medical Research Council War Wounds Committee and Committee of London Sector Pathologists

NOTES ON THE DIAGNOSIS AND TREATMENT OF GAS GANGRENE

With a Suggested Scheme for the Bacteriological Investigation of War Wounds

LONDON
PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE
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York House, Kingsway, London, W.C.2; 120 George Street, Edinburgh 2;
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NOTES ON THE DIAGNOSIS AND TREATMENT OF GAS GANGRENE

With a Suggested Scheme for the Bacteriological Investigation of War Wounds

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I. INTRODUCTION

This memorandum has been prepared by the War Wounds Committee of the Medical Research Council and the Committee of London Sector Pathologists, with the following ends in view :—

(1) To provide a clear and simple synopsis of present knowledge of gas gangrene, based on the clinical and pathological experience of the war of 1914–18, and on more recent experience in Spain, in France and elsewhere.

(2) To indicate in what directions existing knowledge of this condition, and of war wounds in general, most needs to be extended, and how that extension may be most rapidly and effectively secured.

(3) To lay down a detailed scheme for a combined clinical and bacteriological study of war wounds which may be adopted as a minimal procedure by any hospital or bacteriological department willing to co-operate in this investigation.

It is the belief of the Committees that the most hopeful method of obtaining the knowledge required to improve the treatment of gas gangrene and other wound infections is for selected groups of clinical and laboratory workers to devote themselves to the study of particular problems such as are outlined on pp. 8 and 9 below. In conformity with this view, four such groups have already been formed in the London Sector area. It is clear, however, that the intensive study of war wounds, including those incurred in air raids, must depend in large part on an unpredictable distribution of casualties, and it is therefore highly desirable that arrangements should be made in as many areas as possible for studies along similar lines. It is also very important that surgeons and pathologists encountering cases of typical gas gangrene and of lesser degrees of anaerobic wound infection should make careful returns of all such cases upon the Army Form and supplement (p. 8) which have been widely issued for the purpose. The analysis of the data thus obtained should provide a very useful complement to the findings at those hospitals where a more intensive attack upon particular problems is planned.

II. GAS GANGRENE

(*a*) Bacteriology

The causal organisms of gas gangrene are spore-bearing, anaerobic bacilli, belonging to the Clostridium group. The micro-organisms commonly present are, in order of frequency, *Cl. welchii*, *Cl. septique* (*Vibrion septique*), and *Cl. oedematiens*. These bacteria produce their effect by local invasion and intoxication of the tissues, particularly the muscles, leading to necrosis. Generalised invasion by the bacteria occurs only in the more severe cases. All three types, and particularly *Cl. oedematiens*, produce an active toxin. In addition, less pathogenic types of Clostridium may be present, such as *Cl. tertium*, *Cl. sporogenes* and, less frequently, *Cl. histolyticum*, and *Cl. aerofoetidum*. Usually several species of Clostridia occur together in anaerobically infected wounds, but occasionally a single species only may be present. Cases of gas gangrene in which only one species of anaerobe can be found usually yield *Cl. welchii*, and it appears that this organism plays a rôle of major and perhaps primary importance in gas gangrene of the human tissues.

In mixed infections, *Cl. welchii* and *Cl. sporogenes* are commonly found together. Concomitant infections with aerobic organisms, such as Streptococci, Staphylococci, *Proteus vulgaris* (*B. proteus*), *Pseudomonas pyocyanea* (*B. pyocyaneus*) and coliforms are common.

Gas gangrene is a **clinical** conception. It is important to realise that both in the last war and in this, Clostridia have been found, even in large quantities, in infected wounds that have at no time shown any signs of gas gangrene. The clinical diagnosis of gas gangrene may be confirmed by the identification of Clostridia in the wound, especially if they predominate in the specimen or swab, but the disease should not be diagnosed on bacteriological data alone.

The conditions in a wound contaminated with the spore-bearing anaerobes which result on the one hand in a mere local infection and, on the other, in a spreading gas gangrene are not fully understood, but infection is more readily established in wounds containing necrotic tissue. Necrotic tissue provides conditions favourable for the growth of Clostridia. The necrosis may be produced by direct trauma, by more remote interference in the blood supply, or by the action of injurious chemicals and bacterial toxins or of aerobic pathogenic bacteria. Such anaerobic foci are readily produced round foreign bodies like pieces of clothing.

From the bacteriological standpoint, gas gangrene may be controlled in two distinct ways :—(1) *anti-bacterial* (removal of pabulum and introduction of bactericidal substances) and (2) *antitoxic* measures. Antisera capable of neutralising toxins of *Cl. welchii*, *Cl. septique* and *Cl. oedematiens* are available, either singly or in mixture, but it should be realised that these agents have no direct effect upon the bacteria or their invasive power; they are purely antitoxic.

Since the war of 1914–18 knowledge of the Clostridium group of bacteria has advanced considerably. The diffusible toxins are, in many cases, better defined, and more powerful neutralising antitoxic sera are available. Under experimental conditions both *in vivo* and *in vitro*, many of the bacteria are susceptible to the actions of drugs of the sulphonamide group. Experimental data suggest that the most effective control of fully developed gas gangrene with toxaemia may be obtained by the combined use of surgery, sulphonamide chemotherapy, and antitoxins. Recent work by Legroux has shown, moreover, that heavy infections starting round a foreign body introduced into crushed muscle, may be held in check for several days by local insufflation with sulphanilamide powder, and completely eliminated if the insufflation is accompanied by removal of the foreign body. We are still largely ignorant of the precise rôles of organisms of the Clostridium group in the causation of gas gangrene in man; of the parts played by other aerobic and anaerobic organisms; of the relative values of different surgical treatments, and of these treatments combined with chemotherapy, antitoxin, or both chemotherapy and antitoxin. Indications of the best line of treatment will probably come from the records of cases in which therapeutic measures based on a definite plan, or plans, have been carefully checked by repeated bacteriological investigations.

(*b*) Clinical Diagnosis

In a typical case of gas gangrene the onset is acute and the infection progresses with great rapidity—so much so that within a few hours of the onset the patient may be *in extremis*. Cases have been recorded in which established gas gangrene was present within 3¼ hours of wounding. The fact that the onset is delayed, as it may be for as long as 4 to 5 days, by no means diminishes the possibility of a severe infection.

The commonest site of gas gangrene is in muscle. Usually only single muscles or groups of muscles are involved, but the infection may occasionally involve a whole limb or limb segment, especially where there has been interference with the main blood supply. It usually spreads longitudinally up and down the wounded muscles from the site of the lesion, and has little tendency to spread from muscle to muscle. Gas gangrene may also occur primarily in subcutaneous or areolar tissue where there has been extravasated blood: the best example of this is the

very fatal retroperitoneal infection occurring as the result of tangential gun-shot wounds of the abdomen without visceral injury. As indicated in the previous section, lesser degrees of anaerobic wound infection, unassociated with the toxaemia of true gas gangrene are common; such wounds may be foul and exhibit gas bubbles, and from many of them Clostridia may be isolated. The prognosis, however, is relatively benign compared to that in true gas gangrene of muscle.

The most important clinical features of developing or established gas gangrene are :—

Rapid pulse : a rising pulse rate in a wounded man, who has recovered from the initial shock and who is not suffering from continued haemorrhage, is highly significant. The onset of the infection can be differentiated from the rapid pulse of shock by observations on the blood pressure, which is not lowered to nearly the same extent in gas gangrene as in shock; indeed, in gas gangrene the blood pressure may be normal or even slightly raised in the early stages.

Vomiting, although absent in many cases, may be the first suspicious symptom at the onset of the infection. In severe toxaemia vomiting may be a marked feature.

Pain may be a prominent symptom at the onset, as a result of pressure caused by the gas and exudate; it usually ceases when gangrene *en masse* has developed.

Pyrexia is usually present in the early stages; a subnormal temperature is the rule in severe toxaemia.

The general appearance of the patient shows nothing special in the early stages, apart from the flushed face of fever; but as the infection progresses the patient becomes anxious and alert. When toxaemia is severe, the skin becomes " muddy " in colour and may even at times suggest a mild jaundice.

The wound, if of an open type, with exposed muscles, presents the following features. The surface is dry as a whole, though some small amount of thin exudate may escape from beneath the skin edges on gentle pressure; in this exudate gas bubbles may be seen. In the later stages the exudate becomes greater in amount, dark in colour, and extremely offensive. The skin and muscles may show the colour and other changes described later, but these are never so distinctive in an open wound exposed to the air. There is a characteristic smell associated with these wounds, which can be detected before the dressings are removed : this smell is not unlike acetylene gas in low concentration.

When the wound is of the penetrating type, the point of entry being plugged by extruded muscle, the following signs may be present :—

1. *Swelling of the limb :* this is well marked in massive infections and is universal; in infections of single muscles or muscle groups the swelling is much less apparent and may be localised at first to the affected area.

2. *Crepitation* from escape of gas into the subcutaneous tissues through holes in the deep fascia is frequently detectable, but is not constant. Extreme swelling of a muscle prevents the escape of gas through the fascial sheath. It must be realised that the spread of gas bubbles in the subcutaneous tissue, when it occurs, is rapid and that the area involved does not correspond to the area of infection of the deeper tissues. Crepitant subcutaneous tissue is not necessarily infected, and amputation flaps can sometimes be fashioned, with safety, from skin and subcutaneous tissue in which crepitation has been noted. When this is done, however, the flaps should *not* be sutured.

3. *A tympanitic note* on light percussion is nearly always present in cases of massive gangrene, and may be detected on careful examination in the localised and group types of infection if the affected muscles are superficial.

4. *The skin changes* are extremely variable, and it is important to realise that the skin changes do not correspond to the extent of the infection in the underlying muscles; the extent of skin affected is usually less than that of infected muscle tissue, and apparently healthy skin may be lying over seriously infected muscle. In the early stages there are no marked changes in the appearance of the skin, apart

from some blanching around the wound from pressure. As the swelling increases the skin becomes " dirty brown " in colour, with marbling of the surface from stasis in the subcutaneous veins. Mottled purple patches then make their appearance and finally greenish yellow areas, in which blebs may form.

In retroperitoneal infections a peculiar bronzing of the skin overlying the infected area has been described : this may be transient or may go on to extensive destruction of the skin. It may be due in part to disruption of the venules and arterioles running through the loose areolar tissue beneath the skin.

At *operation* certain *muscle changes* may be observed* :—(1) the normal purplish-red colour changes to a brick red, contractility is lost and the cut surface of the muscle does not bleed. Gas bubbles may be seen or crepitation may be felt in the muscle, the fibres of which stand out more prominently and are friable; (2) the brick red colour changes to olive green, the muscle is much more friable and tends to break up on handling; (3) the muscle becomes greenish-black, is glistening and softens to a pultaceous mass.

In flat superficial muscles, such as the sartorius and biceps humeri, the change between normal muscle and infected brick red, non-contractile muscle, can be very clearly seen. The line of demarcation is lighter in colour and a ridge may be palpable between healthy and infected tissue. This ridge is due to the initial swelling of the muscle fibres in the early stages of infection.

In some cases gas bubbles may be seen in X-ray films taken before operation, and this finding may sometimes be of value in recognising the presence of an anaerobic infection. It should be noted, however, that gas may be shown apart from the existence of true gas gangrene, and its extent often has no relationship to the clinical state.

(c) Surgical Prophylaxis and Treatment, with Special Reference to Wound-Excision

Nearly every accidental wound is contaminated by bacteria from the moment of its infliction; of wounds of gun-shot origin this is almost always true. Infection of the tissues bounding and bordering upon the track of the missile almost inevitably occurs after a lapse of time which varies from four or five hours to eighteen hours according to the nature and virulence of the micro-organisms, the amount of damage to the tissues, the loss of blood and the degree of shock and fatigue, all of which contribute to diminish the resistance of the individual to infection. It necessarily follows that excision of the contaminated wound should be practised at the earliest opportunity, before actual infection of the tissues has had time to supervene.

" Through-and-through " wounds, on the other hand, can often be left alone; this applies especially to those which are produced by bullets, and in which there is no evidence of constitutional disturbance. The apertures of entry and exit are often small in such cases; but, even where there is an explosive wound of exit, drainage is free, and excision is usually unnecessary.

In the case of injured men who come under observation only at a later period, when organisms have already reached the living tissues bordering on the cavity of the wound, or when the wound is passing through a " stage of physiological reaction to injury," the chief aim of the surgeon is to provide adequate drainage by appropriate incisions; the time for prophylactic excision has now passed.

In those late cases, however, in which there is any suspicion of gangrenous infection of muscle, the wound must be widely opened up, and the muscle or group of muscles implicated must be ruthlessly excised, whatever be the lapse of time since wounding. Other circumstances may even dictate the desirability of amputation as the sole surgical measure capable of saving life.

* This description of the appearance of the wound, of the muscle changes and the phenomena associated with gas production, applies in the main to *Cl. welchii* infections and to mixed infections to which *Cl. welchii* or *Cl. septique* are contributing. It may be quite inapplicable to the rarer types of gangrene due to *Cl. oedematiens* or *Cl. septique*, where the predominant features may be extensive toxic oedema, with absence of gas, and only an extreme hyperaemia of muscle with no necrotic change.

Technique of Wound-Excision

The wound has probably been protected by some form of first-aid dressing; the region is now exposed, clothes and bandages being cut off, and the skin cleansed over an extensive area around the site of injury. Soap and water will doubtless be required, followed by ether or spirit; some variety of antiseptic solution may be applied to the surface of the skin around the wound.

Skin rarely requires removal and can usually be adequately and efficiently cleaned without recourse to ablation. Should it be necessary to remove any skin at all, a strip not more than a few millimetres wide should be excised. In facial injuries, skin should never be sacrificed.

There must be no hesitation about liberally enlarging the skin-wound in order to secure an efficient exposure of the deeper structures. The necessity for vigorous retraction betokens inadequacy of exposure; anything but the lightest retraction is to be deprecated. Surgical incisions which aim at débridement should be made parallel with the axis of the limb.

The tissues of the wound are now excised with a sharp knife or scissors, particular attention being directed to bruised tags of fascia and to the muscles in proximity to the wound-track. Any alteration in the appearance and colour of the muscle, loss of contractility on mechanical stimulation, or failure to bleed demands wide excision of this most vulnerable tissue. Intermuscular spaces containing blood must be opened up, and blood and blood-clot should be removed, since these constitute an ideal pabulum for bacterial growth. Deep fascia should be freely divided in the long axis of the limb in order to permit subsequent swelling of muscles without strangulation and impairment of blood supply.

Fragments of bone which are completely detached are best removed; they act as foreign bodies, and in the event of infection will indubitably necrose and become sequestra. At the same time, great caution must be exercised not to remove too many loose fragments, lest the future stability of the limb be imperilled. There is a vast difference in prognosis between gun-shot fractures due to mere impact of the missile against the bone and those where the wound-track actually traverses the broken structure; in the former type of injury the risk of infection is trivial if an efficient excision of the soft parts has been performed; in the latter, infection of the bone is inevitable, unless the skeletal injury is thoroughly treated and the medulla adequately opened up. If damaged or doubtful bone requires removal, this must be effected by means of careful and skilful use of a rugine; vigorous tearing at partially detached fragments must never be practised, lest valuable and viable bone be wantonly and needlessly sacrificed.

No excision of a gun-shot wound is complete without the removal of all retained foreign bodies, e.g. fragments of high-explosive bombs or shells, clothing, mud, dirt, etc. The employment of pre-operative radiography will often shorten the time spent over an operation of wound-excision, and reference to an X-ray film in the operating theatre is an invaluable aid to the surgeon in ensuring the removal of all fragments of metal, estimating the nature of damage to bone, etc.

In wound-excision the surgeon must not be oblivious of the future function of the anatomical region with which he is concerned: it is preferable to excise one or more strips of muscle longitudinally, even in the same muscle or group of muscles, rather than to cut ruthlessly across some important muscle, perhaps needlessly sacrificing at the same time much healthy muscle-tissue.

The preservation of important nerve and vascular structures is of the utmost importance, and neglect to act on this principle deserves the gravest condemnation; with adequate exposure these tissues can be thoroughly cleansed. The greatest care must be taken to preserve intact the vessels that remain patent; vascular occlusion may determine non-infective or infective gangrene. Injury to nerves may end in lifelong disability.

In no circumstances should the muscles, fasciae or other deep layers of the wound be sutured with catgut. The propriety of primary suture of the skin should depend upon the experience of the surgeon, the interval between the reception of the wound and the time of operation, the regional distribution and the anatomical characters of the wound, and finally upon the prospect of early transportation. Where the slightest doubt is felt regarding the harmful effect of any of these contingencies, the skin is best left unsutured.

"*Delayed Primary Suture.*" The results of closure of a wound within a few days after excision so closely approximate to those of "primary suture" that this method is one for consideration, when there is the least doubt as to the desirability of "primary suture."

The employment of the sulphonamide drugs, and a growing appreciation of the value of plaster-of-Paris as perhaps the best means of ensuring rest and of facilitating transport, may in the future modify surgical caution in the matter of "primary suture"; but the special experience of the surgeon will count the most.

(*d*) Prophylaxis and Treatment by Serum and Drugs

The anti-gas-gangrene sera so far in common use are antitoxic—that is to say, they neutralise the toxins of certain of the Clostridia, but have no direct action upon the organisms themselves. Laboratory experiments have shown that the sera now available are considerably more protective against the toxins of *Cl. welchii*, *Cl. septique* and *Cl. oedematiens* than those used in 1914–18, but the prophylactic and therapeutic value of these sera for human casualties has not yet been fully determined. Their therapeutic value should theoretically be greatest in cases showing clinical signs of toxaemia.

Supplies of polyvalent serum (containing antitoxins for *Cl. welchii*, *Cl. septique* and *Cl. oedematiens*) and of the three separate antitoxins have been issued. The dispensing arrangements have been based on the dosages mentioned under the headings of prophylaxis and treatment below.* The polyvalent material is dispensed in rubber-capped bottles, each containing one therapeutic dose (plus 20 per cent. excess) or three prophylactic doses of the combined antitoxins; it can thus be used either for therapy, or for prophylaxis where this seems desirable. The monovalent antitoxins are dispensed in single-dose ampoules, each containing one therapeutic dose.

Prophylaxis

It will be realised that prophylactic treatment for gas gangrene means treatment at any time *between the receipt of the wound and the onset of symptoms.* An essential part of such treatment is the surgical procedure of wound excision; the conditions under which this is desirable, and an outline of the method of performing it, have been given above.

Other prophylactic measures include the injection of polyvalent gas-gangrene antitoxin in suitable cases and the use by the mouth and locally of drugs of the sulphonamide group. These measures are specially called for in cases of dirt-contaminated wounds with gross involvement of muscle.

The dosage of polyvalent antitoxin which has been recommended for prophylactic use is as follows:—3,000 international units *Cl. welchii* antitoxin, 1,500 international units *Cl. septique* antitoxin, and 1,000 international units *Cl. oedematiens* antitoxin, given either intravenously or intramuscularly.

The following methods of prophylactic treatment with sulphanilamide or sulphapyridine have been proposed:—

(a) *Oral:* The first dose should be 1·5 grammes (3 tablets) of either compound dissolved in hot 1 per cent. citric acid or hot lemon in order to get rapid absorption. Subsequent doses, starting two hours later and continuing at four-hourly intervals

* The doses at present recommended are provisional and may have to be modified in the light of experience.

for four days, should be 0·5 gramme (1 tablet) as an uncrushed tablet in order to obtain delay in absorption. Dosage: first day 4·5 grammes; subsequent days, 3 grammes; total 13·5 grammes.

Note. If the beginning of treatment has been unduly delayed or if the clinical condition gives reason to fear that gas gangrene is already beginning, therapeutic doses, as below, should be given.

(b) *Local application:* 5–15 grammes of powdered sulphanilamide or sulphapyridine may be packed into the depths of the wound at the time of débridement, or the drug may be blown into the wound with an insufflator, with the object of exerting a bacteriostatic effect. (The local application of the sulphonamide drugs should be used sparingly in the case of wounds involving the brain, as these drugs have been found to act as foreign bodies in contact with nerve tissue.)

Treatment of Gas Gangrene

The surgical treatment of commencing or established gas gangrene has been briefly discussed at pp. 4–6. Recent laboratory experiments (e.g. Henderson and Gorer, *J. Hyg., Camb.*, May, 1940) have indicated that the best available treatment, *in addition to surgery*, for the established disease is probably a combination of antitoxin and of chemotherapy with sulphapyridine or sulphanilamide. The serum and the drug would seem to have a synergic action, the former neutralising the bacterial toxin, while the latter exerts a bacteriostatic effect on the organism itself, thus enabling the body's natural defences to come into play. This combined method of treatment is therefore recommended, and it is very desirable that detailed and accurate records should be kept of cases so treated. The requisite procedure involves administration at the earliest possible moment of gas-gangrene antitoxin in adequate dosage, preferably by the intravenous route, together with full doses of sulphapyridine or sulphanilamide by the mouth or otherwise, as indicated below.

The therapeutic dosage of polyvalent antitoxin which has been recommended is as follows: 7,500 international units *Cl. welchii* antitoxin, 3,750 international units *Cl. septique* antitoxin and 2,500 international units *Cl. oedematiens* antitoxin, given intravenously and repeated as necessary, while symptoms of toxaemia persist. Monovalent antitoxins are available for more intensive therapeutic use in cases where the organism has been identified.

For the therapeutic use of either sulphapyridine or sulphanilamide for gas gangrene, the following dosage is suggested:—

The first dose should be 2 grammes (4 tablets) dissolved in hot citric acid solution or lemon. Subsequent doses, starting two hours later and continuing at four-hourly intervals for two days should be 1 gramme (2 tablets) uncrushed. After the first two days the dosage should be gradually reduced as the clinical condition improves, but the interval between doses should not be more than six hours for several days. Small doses, e.g., 3 grammes *per diem*, should be continued for three or four days after the temperature has come to normal and the clinical condition has become satisfactory. The duration of treatment and the total dosage will vary somewhat, but the latter should seldom exceed 35 grammes.

In cases where the surgeon has to operate upon a wound in which anaerobic infection is already established, pre-operative administration of sulphapyridine or sulphanilamide by the mouth, and of antitoxin intravenously, may be useful in preventing spread of the infection. In these circumstances the first dose of the drug should be 3 grammes, and it should, if possible, be given 2 hours before the operation, so as to secure a high concentration in the blood at the time when the wound is disturbed.

The Need for Further Investigations

It will be realised that much further clinical and bacteriological research on the treatment of gas gangrene and other anaerobic infections is required, the following being some important questions to which answers are needed :—

1. To what extent, and for how long, can bacterial growth be inhibited in wounds locally treated with sulphonamide drugs?

This could be investigated by observations :—

(*a*) on cases where circumstances preclude immediate operation.
(*b*) on cases left open after débridement.

(*Note.*—In laboratories where the necessary biochemical facilities exist, it is important that data should be obtained as to the rate of absorption of sulphanilamide and sulphapyridine from a wound. Such knowledge would avoid the danger of over-dosage in cases where the drug was used both locally as an antiseptic and orally as a chemotherapeutic agent. If absorption from the wound were rapid it might avoid the necessity for coincident administration by the mouth.)

2. Is it practicable to prevent the establishment of anaerobic infection by :—

(*a*) Sulphonamide drugs *per os*,
(*b*) ,, ,, locally,
(*c*) ,, ,, plus serum?

3. Can any correlation be established between the different clinical types of gas gangrene and the infecting organisms (with a view to appropriate serum or drug treatment)?

4. What is the most effective treatment for established gas gangrene and for lesser forms of anaerobic wound infection?

Army Form I 1241 and its supplement, M.R.C. 201, have been issued to facilitate the keeping and return of accurate records of such cases, and it is particularly requested that they shall be completed in sufficient detail to enable the different clinical types of anaerobic wound infection to be differentiated. Exact particulars of the dosage of antitoxin and of drugs used should always be recorded.

III. SUGGESTED SCHEME FOR THE BACTERIOLOGICAL INVESTIGATION OF WAR WOUNDS

By the Committee of London Sector Pathologists

The bacteriological examination of wounds resulting from war injuries is required in the light of modern knowledge :—

(*a*) To provide more precise information about infections so often associated with these wounds, especially the anaerobic infections.

(*b*) To supplement clinical data as to the effects of various methods of treatment upon these infections.

(*c*) To secure further data as to the importance of cross infection by streptococci (and other organisms) in hospital wards; and as to how these may be controlled.

METHOD OF COLLECTION OF SPECIMENS

Owing to the ease with which specimens can be taken on swabs it is recommended that this method be adopted as a routine. The most suitable swab for theatre work is one mounted on a wooden applicator cut to 5 inches in length and wholly enclosed inside a sterile tube plugged with cotton wool. At the operation an attendant can remove the plug and tip out the end of the swab stick, thus allowing the surgeon to take the specimen and replace it in the tube without impairing his sterility. The attendant would then plug the tube, label it, and despatch it to the laboratory.

While the use of a swab is the easiest method, it is some advantage, where possible, for pus to be collected in a test tube or a capillary pipette, as this allows of a more satisfactory microscopical examination.

Specimens should be taken from the deepest parts of the wound.

TIME OF COLLECTION OF SPECIMENS

The first specimen should be collected at the commencement of the operation, in order to ascertain the nature of the primary infection.

Another specimen should be taken at the time of the first complete dressing. Further specimens should be taken at weekly intervals.

This procedure will enable the course of the infection to be ascertained, and the incidence of hospital infection to be determined.

In cases receiving special methods of treatment (e.g. by the sulphonamide drugs) specimens should be taken more frequently, and the treatment be checked by as detailed bacteriological examination as possible, following the methods outlined below.

PROCEDURE

1. *Microscopic examinations of films of material from the wound.* Gram stained films should always be examined. They may give immediate information to the surgeon as to the general nature of the infection, and also indicate to the bacteriologist suitable special methods of culture.

2. *Cultural methods.* It is recommended that the following cultures be made :—

(*a*) Aerobic blood-agar plate of unheated pus.

(*b*) Anaerobic blood-agar plate of unheated pus (incubated in McIntosh & Fildes jar).

The anaerobic plates should be well dried before incubation, to prevent the undue spreading of anaerobic colonies.

An indicator tube should always be placed in the anaerobic jar (see Appendix 6).

(*c*) Litmus milk for the detection of *Cl. welchii*. The milk tubes should be boiled to expel any air; and, on cooling, they should be thickly inoculated from the

swab or pus, covered with melted vaseline and incubated without further anaerobic precautions.

(*d*) Robertson's meat medium. This grows both aerobes and anaerobes, and is especially useful in that it provides a culture to which the bacteriologist can return if for any reason his plate cultures are unsuccessful. It also allows slowly developing anaerobes to be detected in subcultures made after some days' incubation. These cultures, if they are to be stored, should be covered with a layer of liquid paraffin to prevent drying.

Note 1. It is recommended that the litmus milk and the meat media be prepared in 6 × $\frac{5}{8}$ inch test tubes, in preference to screw-capped bottles.

Note 2. If in any laboratory it is impossible to proceed with the isolation of anaerobes, a meat tube should be inoculated from the wound swab and sent to a reference laboratory.

Anaerobic Plate Cultures

These will yield many aerobic organisms, both spore-bearers and non-spore-bearers, in addition to the obligate anaerobes. It should be remembered that :—

1. Some strains of bacteria which first appear exclusively on the anaerobic plate will prove, on subculture, to be aerobes.

2. Colonies of some aerobic spore-bearers, when growing anaerobically, will often simulate those of Clostridia.

3. The cultures should be examined the day after inoculation and again after 48 hours, as the colonies of the spore-bearing anaerobes may then be more characteristic.

Spore-bearing anaerobic bacteria

The following table may be useful as giving some of the more important characters of the common members of this group :—

Organism	*Surface colonies*	*Deep colonies in agar*	*Litmus milk*	*Coagulated serum or egg*	*Spores*
Cl. welchii ..	Large, regular probably hæmolytic.	Lenticular, opaque.	Stormy acid clot.*	—	Seldom seen in culture.
Cl. œdematiens ..	Clear, slightly irregular.	Woolly	Acid	—	Central and sub-terminal. Very few.
Cl. septique (*Vibrion septique*).	Transparent, spreading.	Transparent, branching.	Little change (? slight acid).	—	Central or sub-terminal.
Cl. sporogenes ..	Medusa-head (if plate dry); irregular (if plate moist).	Woolly, opaque.	Digestion with foul odour.	Digestion	Central or sub-terminal.
Cl. tertium ..	Small, clear, circular.	Small, lenticular.	Little change.	—	Terminal oval.
Cl. tetani	Very transparent, spreading.	Delicate filaments.	Little change.	—	Terminal round.

* This appearance may be simulated by certain other microbes.

Pathologists who wish to carry out further differentiation of their strains are advised to consult a standard textbook.

Note 1. If fermentation tests are made, it should be noted that some indicators, such as Andrade's, may be irreversibly decolorised in the anaerobic jar. Tubes showing no apparent increase in acidity after anaerobic incubation should be tested with a drop or two of fresh indicator.

Note. 2. *It is emphasised that the presence of Cl. welchii in a wound is not necessarily an indication of gas gangrene; this organism is present in the cavities of many wounds of patients not suffering from that disease.*

Note 3 (*Streptococci*). Observations on wounds in the present war have confirmed past experience that streptococci may be found in anaerobic cultures which do not appear on aerobic blood agar plates. While some of these are true anaerobes, many are cocci which, when first isolated, will not grow aerobically but which soon become acclimatised and grow readily in open tubes and plates.

The true anaerobic streptococci should be investigated as to cultural reactions—digestion of albumen, production of foetid odour, sugar reactions—and as to pathogenicity for animals. Where possible, cultures should be maintained in Robertson's meat medium for future study. It is suggested that a microscopical examination of the original meat culture should be made after some 3 days incubation (this also gives valuable information as to the spore-bearing anaerobes present), and if streptococci are seen which did not appear in the culture plates, further cultures should be made on blood-agar anaerobically. Streptococcal colonies which appear may then be subcultured into "sloppy" glucose-agar (glucose-broth containing 0·1—0·2 per cent. agar).

Aerobic Plate Cultures

Haemolytic streptococci: These should be isolated and tested for soluble haemolysin (for method, see Appendix 1). If soluble haemolysin is present, aerobic or anaerobic cultures may be sent for grouping and typing to:

Dr. F. Griffith,
Research Laboratory for Streptococcal Infections,
Ravenscourt Square, London, W.6.
(*Telephone No.:* Riverside 2174.)

Staphylococci: These should be isolated and the coagulase test performed (for method, see Appendix 2).

Other organisms: The presence of other organisms should be recorded.

Relative numbers of different bacteria present. From the primary microscopical examination, and from the aerobic and anaerobic cultures, some indication of the *relative numbers* of the different bacteria present should be recorded in all cases.

Records. It would be an advantage if in each laboratory a special book were kept for recording cases of wound infection; in this, in addition to the bacteriological findings, there should be noted for very case:—

(*a*) The nature of the wound.
(*b*) The time after injury that each swab was taken.
(*c*) Particulars of therapy, local and general.

APPENDIX 1

Method of Detecting Soluble Haemolysin

The streptococcus is grown in 15 per cent. serum broth for *not more* than 15 hours (cultures may be put in the incubator last thing at night and tested first thing next morning). Equal volumes of the culture and 5 per cent. washed horse corpuscles are mixed and incubated in a water-bath at 37° C. Haemolysis with group A streptococci usually occurs within 15 minutes. Final readings are made in one hour. Complete haemolysis is produced by Groups A, C and G. Haemolytic Group B streptococci tend to give incomplete haemolysis.

The washed corpuscles will keep well in a refrigerator for several days.

APPENDIX 2

Coagulase Test for Staphylococci

Of an overnight broth culture add 2 drops (0·07 to 0·1 cc.) to approximately 1 cc. of citrated plasma diluted 10 times in normal saline (human or rabbit plasma may be used). Incubate in a water bath at 37° C. If the test is positive an obvious coagulum usually appears in 1 or 2 hours, but incubation can be prolonged for 6 hours.

The citrated plasma keeps well and can be diluted in saline as required.

APPENDIX 3

The Use of Potassium Tellurite as a Selective Bacteriostatic Agent in Culture Media

The bacteriostatic properties of potassium tellurite on nutrient agar are approximately as follows:

Haemophilic bacilli and most coliforms are inhibited by a concentration of 1 in 500,000.
B. pyocyaneus is inhibited by a concentration of 1 in 50,000.
B. proteus is variable and many strains are resistant to 1 in 50,000.
Streptococci, staphylococci, and diphtheroid bacilli are resistant to 1 in 10,000.

If, in the direct microscopical examination of the pus, large numbers of coliform organisms are seen, potassium tellurite may be incorporated in the medium in a concentration of about 1 in 50,000, in order to inhibit them and to allow the cocci to be easily isolated. A much simpler method, however, is to inoculate a plate in the usual way, and then to spread 2 or 3 drops (60 to 100 cmm.) of 1 in 1,000 tellurite over half of the plate. In this way one half of the plate is an ordinary culture, while on the other half the coliforms are generally completely inhibited.

A convenient stock solution is 1 in 1,000.

Potassium tellurite may be used in the same way for the separation of anaerobes, as there are differences in the sensitivity of different members of this group to the chemical. More work, however, remains to be done in order to determine its exact usefulness in this direction.

APPENDIX 4

Negative Staining for the Detection of Clostridial Spores in Culture

For this purpose a solution of Gurr's Nigrosin in water about three quarters saturated can be used.

A small drop of the Nigrosin solution is placed on a slide, and some of the culture is mixed with this and spread out into a thin film by means of a wire or another slide. This film is allowed to dry, and can then be examined. In the thicker parts of the film the large spores stand out as clear spots, while the bacillary portions are partly overlaid by the nigrosin.

An alternative method is to make a film of the bacteria in water on a slide in exactly the same way as is done preparatory to staining. When this film is dry a small drop of nigrosin is placed on the slide and is spread in a thin film over the bacteria.

The great advantage of this negative staining method is its simplicity; and, if necessary, confirmation of the existence of spores can be obtained by the usual staining methods.

APPENDIX 5

Isolation of Organisms from Material containing B. proteus

The following technique is suitable. A blood-agar plate is inoculated in the usual way, and then melted agar at 45° C. is poured over the surface of the plate to a depth of 2 or 3 mm. and allowed to set. After incubation it will be found that any colonies of *B. proteus* growing between the two layers of agar show no tendency to spread, and colonies of other organisms can be easily picked out from among them. It generally happens, however, that some of the proteus spreads round the edge of the agar on to the upper surface; and this must be killed before any deep colonies are picked out. A satisfactory method is by flooding the surface of the plate with saturated mercuric chloride solution for about thirty seconds. The solution is washed off with tap water, and some of the surface growth of proteus is scraped off with the end of a microscope slide, to enable the deep colonies to be examined. These can then be picked out and subcultured.

As an alternative, the surface growth of proteus can be removed by scraping with a slide and washing with tap water, and then the surface can be sterilized by flooding it with boiling water.

In suitable cases potassium tellurite in concentrations of 1 in 20,000 to 1 in 50,000 can be incorporated in the agar which is poured over the surface. This inhibits most of the coliform bacilli, and sometimes prevents *B. proteus* from spreading over the surface.

APPENDIX 6

Indicator Tube for Anaerobic Jar

To a tube of 5 cc. of 2 per cent. glucose-broth add 0·1 cc. of Loeffler's alkaline methylene blue. This should become decolorised in the jar, and it should remain colourless throughout incubation if anaerobic conditions are maintained.

APPENDIX 7

Influence of CO_2 on the Growth of Bacteria in Wounds

It has been shown that some streptococci and other bacteria will not grow, or will grow only poorly, unless there is an increased amount of CO_2 in the atmosphere. Where facilities exist, observations should be made on the effect of an atmosphere of from 2 to 10 per cent. of CO_2 on the growth of aerobes and anaerobes from war wounds. A simple technique for this purpose is described in Memorandum No. 5 of the Department of Bacterial Chemistry, Medical Research Council, and by Gladstone and Fildes, *Brit. J. exp. Path.*, August, 1940.

It consists in using tin containers, 6″ × 8″, of capacity 3,600 cc., with " press down " lids. CO_2 is generated in the tin from marble and HCl. The cultures, either plates or tubes, are inserted together with an open tube 8″ × 1″ containing 8 cc. (excess) of 25 per cent. HCl. A marble chip of about 0·7 gramme is dropped into the acid and the lid pressed down. This is calculated to give approximately 5 per cent. concentration CO_2 at 37°, but, since the exact concentration is unimportant, the weight of the marble need only be very approximate. The increased pressure due to the CO_2 is not important. Further cultures may be added without using fresh marble and acid, provided that this is done reasonably quickly.

Printed under the authority of His Majesty's Stationery Office
By Eyre and Spottiswoode Limited, London

(382)22435 Wt 1878—831 9000 9/40