Notes on gas gangrene : prevention, diagnosis, treatment with an account of the technique of wound-excision and a scheme for the bacteriological investigation of war wounds / by the War Wounds Committee of the Medical Research Council and the Committee of London Sector Pathologists.

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NOTES ON GAS GANGRENE

PREVENTION
DIAGNOSIS : TREATMENT

With an Account of the Technique of Wound-Excision and a Scheme for the Bacteriological Investigation of War Wounds

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

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CONTENTS

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.

(2) To indicate in what directions existing knowledge of this condition, and of war wounds in general, most needs to be extended, and how additional information may be most 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. 17 and 18–24 below. In conformity with this view, four such groups were formed in the London Sector area during the period of heavy aerial bombardment in 1940–41. 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* I.1241 *and its supplement* (I.1241A) *which, with corresponding E.M.S. forms, have been widely issued for the purpose* (*see below*). *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) General Considerations

Nearly all war wounds, particularly those caused by bomb or shell splinters, are contaminated by bacteria from the moment of infliction. Infection of the tissues bordering upon the track of the missile almost inevitably occurs after a lapse of time which varies from three 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 diminishing the resistance of the individual to infection. Gas gangrene is one of the most serious complications of war wounds; it develops usually within 24 to 48 hours after wounding, though the onset may sometimes be much more rapid and sometimes delayed for many days.

Gas gangrene is due to infection of tissues, particularly muscle, with anaerobic bacteria of the Clostridium group, the main infecting organisms being *Cl. welchii* (B. perfringens), *Cl. oedematiens* (Cl. novyi) and *Cl. septicum* (vibrion septique). It is, however, a clinical condition and does not mean simply the presence of gas-producing bacteria in the wound. It is important to realise that Clostridia are frequently found in wounds which at no time show any sign 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 characteristic features of gas gangrene are due to the fact that, as in diphtheria and tetanus, the infecting bacteria produce soluble toxins which diffuse into the tissues and ultimately cause toxaemia. But whereas in diphtheria and tetanus the bacteria remain localised near the site of infection, in gas gangrene they invade the healthy tissue surrounding the wound and continue to multiply. This invasion depends on the power of the toxins to damage the tissue cells and pave the way for the penetration of bacteria. As the organisms multiply, they produce more toxin, which in turn further increases the area of damaged and necrotic tissue, so that the infection may spread rapidly from a wound of comparatively small size. General signs of toxaemia may develop as the toxins are absorbed, and the toxic effect may persist even if the bacteria die out, since the toxin once formed is independent of the bacterial cell.

The problem of preventing gas gangrene or controlling the established disease is therefore twofold. The primary necessity is to check the bacterial growth and stop the production of toxin. This may be done by early excision of the wound, which removes infected and necrotic tissue, and also by the use of drugs which kill the bacteria or prevent their multiplication. Secondly, any toxin formed should be neutralised before it can cause further damage locally or lead to a general toxaemia; this can be achieved by ensuring the presence in the blood of an adequate amount of the antitoxin to the particular toxin present.

Recent research has indicated that the combined use of surgery, antitoxin and bacteriostatic drugs should prove the most effective means available for preventing or treating gas gangrene. The importance of prophylaxis against a disease which has a mortality rate of about 50 per cent. and frequently involves the amputation of a limb need hardly be emphasised, even though the incidence of the disease in the present hostilities has so far been low. During the war of 1914–18 the introduction of prompt and adequate wound excision reduced the incidence of gas gangrene in casualties from approximately 10 to 1 per cent. or less. In the present war, surgical treatment of casualties has often been unavoidably delayed for hours and even days; consequently, prophylaxis with antitoxin and drugs, which can be carried out in advanced field units, is of special significance. It is known that the simultaneous use of antitoxin and sulphonamide drugs effectively prevents certain types of experimental gas gangrene in animals, but its value in preventing gas gangrene in man, which is commonly due to a mixed infection, has yet to be assessed. These measures are not a substitute for wound excision, but their prompt use offers at present the best chance that the infection and the toxaemia will be kept under control until surgical intervention is possible. Even when early wound excision is possible, antitoxin and

sulphonamide drugs should be used, to ensure the maximum protection attainable in the light of existing knowledge.

Records.—The value of these prophylactic methods, or of any line of treatment of the established disease, can be proved only by the analysis of careful records of cases in which the measures taken have been based on a definite plan and checked bacteriologically. Army Form I. 1241 and its supplement I. 1241A, and the corresponding E.M.S. forms, have been issued to facilitate the keeping and return of accurate records of cases of gas gangrene. It is particularly requested that these forms shall be completed in respect of *all* cases of established or suspected gas gangrene, in sufficient detail to enable the different clinical types of anaerobic wound infection to be distinguished; exact particulars of the dosage of antitoxin and of drugs should always be recorded. It should be noted that in Army Form I. 1241, paragraphs 4 and 5 headed " prophylactic treatment " and " surgical intervention " respectively are intended to refer to prophylaxis against gas gangrene undertaken *before anaerobic infection is clinically apparent.* The details of such treatment will frequently have to be copied from the Field Medical Card (Army Form W.3118).

(b) Causes of the Disease

The causative agents of gas gangrene are strictly anaerobic spore-bearing bacilli of the Clostridium group. Usually several species of Clostridia occur together in a wound, but occasionally only a single species may be present. The chief species causing toxaemia are *Cl. welchii, Cl. oedematiens* and *Cl. septicum*; the toxins of *Cl. welchii* and *Cl. septicum* have a destructive action on the tissues which favours the spread of infection; *Cl. oedematiens* is relatively non-invasive, but it liberates a potent toxin.*

In the war of 1914–18, the pathogenic species isolated from cases were, in order of frequency, *Cl. welchii, Cl. septicum, Cl. oedematiens.* In Libya in the present war, the majority of cases have been infected with *Cl. oedematiens*, either alone or in conjunction with *Cl. septicum* or *Cl. welchii.* All these organisms are found in soil; *Cl. welchii* is usually present in the intestinal tract in man and lower animals.

Other species of Clostridia which may be present in wounds include *Cl. tetani* and the relatively non-pathogenic species, *Cl. tertium, Cl. sporogenes, Cl. histolyticum, Cl. bifermentans* and *Cl. fallax.* Concomitant infections with aerobic organisms, such as streptococci, staphylococci, *Proteus vulgaris, Pseudomonas pyocyanea* and coliform bacteria, are very common.

The conditions in a wound contaminated with the spore-bearing anaerobes which result, on the one hand, in a mere local infestation and, on the other, in a spreading gas gangrene infection are not fully understood. Gas gangrene is more readily established in wounds containing necrotic tissue, which provides conditions favourable for the growth of Clostridia. The necrosis may be produced by direct trauma or by remote interference with the blood supply, or by the action of injurious chemicals or bacterial toxins. Foreign bodies, such as pieces of clothing or metal, readily act as foci of infection. The development of the disease may be assisted by the presence of aerobic and other anaerobic organisms which, by damaging the tissues—for example, by proteolytic action—favour the growth of strict anaerobes.

The local and general symptoms of anaerobic wound infection vary greatly in different cases, because of the many factors involved. Such variations in the severity of the effects may depend partly on the actions of the specific toxins of the infecting organisms and partly on the site of the wound, which influences the speed at which the toxin reaches the circulatory system. Indications of the best line of treatment in different types of case will come through records in which therapeutic measures based on a definite plan have been carefully checked by repeated bacteriological examinations.

(c) 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

* A more detailed account of the characteristics of these organisms is given in Section III (pp. 20–23).

may be *in extremis*. The time of onset is variable, though usually it is within 24 to 48 hours after wounding. On the other hand, cases have been recorded in which established gas gangrene was present within 3¼ hours of wounding, or the onset may be delayed for 4 to 5 days or even longer; a delayed onset 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 muscle 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 with that in true gas gangrene of muscle.

It is essential that the surgeon should be able to recognise the conditions under which gas gangrene is particularly liable to develop, and the early clinical symptoms and signs of the disease. The possibility of gas gangrene should be borne in mind:—

1. Where there has been extensive laceration of muscles—e.g. in compound fractures of the long bones, especially if the arterial blood supply has been affected. (It should be remembered that insignificant entry wounds made by bomb or shell fragments may be associated with extreme muscle laceration, especially if the entry wounds are multiple. Gas gangrene infections are more likely to occur in wounds of the lower limb and buttock than in any other part of the body.)
2. Where the wound is obviously contaminated with soil, dust or rubble, or it is probable that fragments of clothing or other contaminated foreign bodies have been carried deeply into the tissues.
3. Where a tourniquet or tight bandage has been applied for a long period, the wound has been tightly packed or there is injury to the main arterial supply to the affected part.
4. Where there is severe shock and operation has to be postponed for many hours.
5. Where, in patients with wounds of any of the types indicated above, difficulties of evacuation or transport will inevitably cause delay in surgical treatment.

While experience has shown that the majority of gas gangrene infections develop under these conditions, it should be realised that, owing to the many variables discussed in the preceding section, gas gangrene may occur unexpectedly in other cases. Thus, a patient with an extensive wound and much damage to muscle may make a good recovery without evidence of toxaemia, while one with a small wound and relatively little local necrosis may succumb rapidly to a severe toxaemia.

The signs of established gas gangrene are definite and are given below, but it is important that medical officers in charge of wounded men should know the premonitory symptoms and signs, so that they may diagnose the disease, and take appropriate therapeutic steps, before it has fully developed. The earliest symptoms and signs may include:—

1. Increasing pain in the wound area with, possibly, the appearance or increase of local swelling.
2. A rising pulse rate in the absence of continued haemorrhage and after shock has been overcome.
3. A change in the mental attitude of the patient, which is difficult to define with accuracy: this change may be one of mental apathy or sometimes euphoria, with or without restlessness.
4. A wound which is relatively dry or, at most, shows a thin discharge, the exposed muscle and fat at times being stained a plum colour from haemolysis.

Occasionally, unexplained attacks of vomiting may occur in the early stages.

In other instances, the presence of previously undetected gas gangrene infection may be determined at operation by observing early changes in the muscle (see below).

An immediate microscopic examination of the wound exudate in a suspected case should be made whenever possible; it may yield useful information about the general nature of the infection (see p. 18).

The most important features of established gas gangrene are:—

Rapid pulse: a rising pulse rate in a wounded man, who has recovered from the initial shock and is not suffering from continued haemorrhage, is highly suggestive.

Vomiting may occur early or may be absent; it may be a distressing symptom in patients with severe toxaemia.

Pain may be a prominent feature at the outset, 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 droplets of fat and gas bubbles may be seen, and a smear may show large numbers of Gram-positive rods, some spore-bearing, with few or no pus cells. 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 generally be detected before the dressings are removed: this smell is not unlike acetylene gas in low concentration.

In certain types of wound, where the point of entry is 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 skin and subcutaneous tissues are not necessarily infected.

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 white 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 be of value in supporting the diagnosis of an anaerobic infection. It should be remembered, however, that gas may be shown apart from the existence of true gas gangrene, and that its extent often has no relationship to the clinical state.

(d) Prophylaxis of Gas Gangrene

Prophylaxis means the carrying out of measures which tend to prevent a disease, and, strictly speaking, it covers any treatment given to the patient before the disease is recognisable. It therefore includes treatment given before there is risk of infection and also treatment given after it is known that infection may have taken place. It is essential to realise, however, that prophylaxis given after the injury has been received should be carried out as soon as possible. This is particularly important in regard to gas gangrene, as the incubation period of the disease may be less than four hours, though usually it is 24 to 48 hours. At some time during this incubation period the condition ceases to be merely a contamination of damaged tissue with gas-forming bacteria, and becomes an active, though not necessarily a manifest, gas gangrene infection, and there are no means of deciding exactly when this crucial change occurs. Prophylactic measures are most effective if carried out early in the incubation period; this means, in practice, as soon as possible after wounding.

There are three major measures available at present for the prophylaxis of gas gangrene. The first two, surgical excision of the wound and the application of bacteriostatic drugs, are aimed, respectively, at eradicating the contaminated area and at preventing bacterial growth. The third measure, administration of antitoxin (passive immunisation), is aimed at neutralising the toxin produced by the bacteria. These measures are complementary to each other and should be used in conjunction whenever possible. The mechanical removal of infected tissue by operation is most important, but sulphonamide drugs reduce the risk of subsequent growth of bacteria in inaccessible pockets, or may hold the infection in check if immediate operation is impracticable or dangerous. Neutralisation of the toxin by the antitoxin will prevent or minimise toxaemia, and, since some of the toxins themselves cause tissue necrosis, this neutralisation indirectly checks the spread of infection.

It has been shown experimentally that the simultaneous use of gas gangrene antitoxin and of sulphonamide drugs has a synergistic effect, i.e. the combined effect is greater than the sum of the two effects acting independently; the check given both to the action of toxin and to the bacterial growth apparently gives sufficient time for the natural defences of the body to be mustered and to overcome the infection. This emphasises the advisability of using all three methods—surgery, antitoxin and bacteriostatic drugs—in the prophylaxis of gas gangrene, and since both the antitoxin and the drugs undergo elimination from the body, it will be necessary in some cases to repeat the dosage (see below).

Antitoxin and sulphonamides can be administered in the forward areas, whereas surgical excision must await the arrival of the patient at a point where operative

* 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. septicum* are contributing. It may be quite inapplicable to the types of gas gangrene due to *Cl. oedematiens* or *Cl. septicum*, where the predominant features may be extensive toxic oedema, with absence of gas, and only an extreme hyperaemia of muscle with no necrotic change.

facilities are available. The prophylaxis of gas gangrene is, therefore, dealt with in this chronological order, but it is to be understood that surgical treatment of the wound is of the greatest importance and must never be unnecessarily delayed.

(i) Prophylactic Use of Antitoxin

Production and mode of action of antitoxin.—The soluble toxin obtained from a bacterial culture is changed by treatment with formalin into a non-toxic derivative (*toxoid*), which, like the original toxin, is *antigenic*, i.e. on injection into animals it stimulates the production of a specific antibody which appears in the blood as part of the serum proteins. This antibody can combine with the toxin. The antibody produced as the result of inoculation with a toxin or toxoid is an *antitoxin*, and the serum in which it is present is known as an antitoxic serum. The serum obtained after immunisation with, for example, *Cl. welchii* toxoid, neutralises the toxin of *Cl. welchii*, but not the toxins of other bacteria; the antitoxin produced by immunisation with *Cl. septicum* or *Cl. oedematiens* toxoid is similarly specific against the corresponding toxin. The antitoxins have no direct effect upon the bacteria themselves.

The presence of antitoxin in the blood of man may be secured in two ways: (*a*) by *active* immunisation, i.e. by inoculating the subject with the corresponding toxoid, or (*b*) by *passive* immunisation, i.e. the injection of antitoxin.

On active immunisation, the antitoxin level in the blood rises in the course of a few weeks to a maximum and then decreases gradually to a low level which may be maintained for many months. Once this basal immunity is established, the response to a subsequent dose of toxoid is, in the majority of cases, much more rapid than to the initial inoculation, though several days elapse before there is an appreciable rise in titre. On the other hand, by passive immunisation, through the injection of antitoxin, the level in the blood is raised immediately, but as the greater part of this antitoxin is eliminated in the course of a week or so, the immunity must be renewed or augmented, if necessary, by further doses.

Active and passive immunisation are both useful in prophylaxis. For example, tetanus toxoid is injected to establish a basal immunity to tetanus before the subject is exposed to the risk of being wounded. A British casualty also receives a prophylactic dose of tetanus antitoxin as soon as possible after wounding, to reinforce this immunity. The practicability of active immunisation with gas gangrene toxoids is under investigation, but at present only passive immunisation, by injection of gas gangrene antitoxins, is possible.

Antitoxic sera are produced by the immunisation of horses. The antitoxin is present in the globulin fraction of the serum proteins, and is concentrated and separated from the albumin by precipitation with salts. The antitoxic globulin can be further purified by treatment with pepsin, and is then known as *refined* (pepsin-treated) antitoxin. The polyvalent gas gangrene antitoxin issued is a mixture of the concentrated antitoxins to *Cl. welchii*, *Cl. oedematiens* and *Cl. septicum* toxins. The potency of these antitoxins is determined by comparative tests carried out in relation to the International Standards, and is expressed in International Units.

Use of antitoxin.—A prophylactic dose of polyvalent gas gangrene antitoxin should be given as soon as possible after wounding to all casualties of the types indicated on p. 6 as being particularly liable to develop gas gangrene; it is especially called for in cases of dirt-contaminated wounds with severe laceration of muscle, in cases where contaminated foreign bodies may have been carried deeply into the tissues, or where there is interference with the main blood supply to a limb. The dose of polyvalent antitoxin recommended for prophylaxis is 9,000 units *Cl. welchii* antitoxin, 4,500 units *Cl. septicum* antitoxin, 3,000 units *Cl. oedematiens* antitoxin.*

Intravenous injection is the best method to secure a rapid increase in circulating antitoxin, and it should be used whenever possible.† If direct venepuncture is diffi-

* It may be noted that the prophylactic dosage of antitoxin recommended in the first edition of this pamphlet was one-third of this amount, but the dosage has latterly been increased upon the recommendation of the Anaerobes Sub-Committee. The dosage is still provisional, and the possibility of further increasing the unitage of *Cl. oedematiens* antitoxin in the polyvalent mixture is under consideration.

† The Canadian and United States military authorities do not favour the intravenous injection of prophylactic antisera, but this route is recommended in British practice.

cult, the vein should be exposed for injection, as the collapse of veins is frequently due to the action of the toxins, which should therefore be neutralised as quickly as possible. Under conditions where intravenous injection is impracticable, such as in advanced field units, the prophylactic dose should be given intramuscularly into healthy tissue. After intramuscular injection, antitoxin can be detected almost immediately in the blood, and the level rises gradually to a maximum at 36 to 48 hours. There would seem to be no particular advantage in injecting antitoxin into the region immediately around the wound.

Repetition of dose.—The level of antitoxin declines slowly from the maximum, but is still appreciable a week after injection in the healthy subject. Since this time covers the usual incubation period of gas gangrene, a second prophylactic dose need not be given as a routine. It should be remembered, however, that if, in fact, there is an active infection of the wound with production of toxin, the antitoxin will disappear more rapidly through combination with the toxin, and the signs of toxaemia may be masked. The clinician should use his own judgment about giving a second prophylactic dose of antitoxin at any time, before or after operation, if the condition of the patient or of the wound is in any way suspicious.

Reactions following the administration of antitoxin may be immediate or delayed. The immediate reactions, occurring within two hours of injection, include thermal (rigors, with headache or malaise, with or without a sharp rise of temperature) cardiovascular (circulatory collapse) and allergic effects (rashes, joint effusions, anaphylactic shock). The commonest delayed reaction, which is also allergic in origin, is serum sickness, usually occurring 7 to 10 days after administration. The risk of severe anaphylactic shock following antitoxin is small, especially if refined (pepsin-treated) antitoxin is used. A syringe ready charged with 1 : 1,000 adrenaline hydrochloride (Liquor Adrenalinae Hydrochloridi, *B.P.*) should, however, always be at hand for the treatment of severe allergic reactions if they occur (dose: 5–15 minims; 0.3–1.0 c.c.).

(ii) Prophylaxis with Bacteriostatic Drugs

Under conditions of mobile warfare, it may often happen that adequate surgical treatment has inevitably to be delayed for many hours, and in such cases the use of sulphonamide drugs (and of antitoxin) as soon as possible after wounding may contribute largely to the saving of life and limb. To ensure a sufficient concentration of sulphonamide in the neighbourhood of the contaminating bacteria, a drug of this group should be applied locally to the wound, as well as being given by mouth to the more severely wounded.

(a) *"First-Aid" Application of Sulphonamides in the Field or at Aid Posts*

For the first-aid treatment of wounds by medical officers in the field or at an aid-post, the application of sulphanilamide powder is generally recommended. Sulphanilamide, being the most soluble of the sulphonamide drugs, will quickly set up a high sulphonamide level in the fluid bathing the injured tissues of open or superficial wounds to which it is applied.

The amount of sulphanilamide powder to be applied locally will depend upon the surface area of tissues to be covered. In general, 5–10 gm. will be sufficient for a single large wound, and it will seldom be wise to use more than 15 gm. in all. The powder should be thinly sprinkled or dusted over the wound surface.

While the local use of sulphanilamide powder as indicated is suitable for the treatment of superficial wounds and those with a free exposure of damaged tissue, it is doubtful whether it will be effective in small-aperture penetrating wounds or in wounds with deep recesses. In such cases, the application of fluid preparations which can be injected through the sterile nozzle of a collapsible tube may be preferable. A sterilised 15–20 per cent. microcrystalline suspension of sulphathiazole is under clinical trial for this purpose, but it is not yet generally available. It should be injected only by medically qualified personnel, and must not be applied in the immediate neighbourhood of important nerves. The contents of one tube should be sufficient for an average severe wound.

In patients with severe wounds, where the risk of gas gangrene or of septic

infection is believed to be great, the local application of sulphanilamide powder (or of sulphathiazole suspension) should be supplemented by oral administration of sulphathiazole, sulphapyridine, sulphadiazine or sulphanilamide in the following prophylactic dosage, starting as soon as possible after wounding:—

Initial dose: 4 gm. (= 8 × 0·5 gm. tablets).
Second dose (2 hours later): 1 gm.
Third dose (4 hours later): 0·5 gm.
Continue doses of 0·5 gm. 4-hourly thereafter for 4 days.

The dosage—or the spacing of the doses—will need adjustment where operation, with a thorough local application of sulphonamide, takes place during the course (see below).

Absorption of the drug will be accelerated if the tablets are crushed or chewed, before being swallowed with a drink of water. A generous fluid intake (about 6 pints in 24 hours) should be ensured if sulphathiazole, sulphapyridine or sulphadiazine is given, owing to the risk of renal complications (p. 16). This is particularly important in hot climates, where sweating may be profuse.

(b) *Application of Sulphonamides to Wounds at Operation*

Whether or not a sulphonamide drug has been applied earlier to the wound, an application of sterilised sulphanilamide or sulphathiazole powder (or of a mixture of 3 parts of sulphanilamide to 1 part of sulphathiazole) should be made at operation. The mixture has the advantage of maintaining the local concentration of sulphonamide for a longer time, and sulphathiazole has been shown experimentally to be more active than sulphanilamide against the organisms of gas gangrene (see p. 15). The technique of applying the powder is included in the description of the operation of wound-excision (below). When a thorough local application of sulphonamide has been made to the wound at operation, none should be given systemically during the first 12 to 24 hours afterwards, unless premonitory symptoms of gas gangrene indicate an urgent need to begin intensive therapy (see p. 16); in such cases, half the amount applied locally should be deducted from the total to be given systemically on the same day.

In patients in hospital the effects of administering sulphonamides locally and systemically should, when possible, be controlled by estimations of the concentration of the drug in the blood, and frequent leucocyte counts should be made, to guard against the risk of agranulocytosis (see p. 16).

Sulphonamide drugs, in general, should be applied only sparingly to wounds involving the brain; sulphathiazole, in particular, should never be applied to brain wounds, as it has been shown to cause epileptiform convulsions.

(iii) Surgical Treatment of the Wound, with special reference to Wound-Excision.

The most important of all methods of prophylaxis against gas gangrene is the excision of the contaminated wound at the earliest opportunity, before actual infection of the tissues has had time to supervene. The chances of success with surgical treatment are increased by the prophylactic use of antitoxin and drugs (see above), but these measures must be regarded as ancillary to adequate surgery, and not as replacing the need for it. While the use of these adjuncts to surgery may extend the period between the time of wounding and surgical aid after which a formal "surgical revision" (excision) may still be wisely and advantageously performed, it can never justify unnecessary delay in operative procedure. Under conditions where a formal excision of the wound is impossible, it is justifiable to enlarge the skin wound under local anaesthesia and to incise the deep fascia, so as to relieve tension and provide free drainage: sulphanilamide or sulphathiazole (or a mixture of the two) may then be applied to the interstices of the enlarged wound (see above).

"Through-and-through" wounds, alone among war wounds, can sometimes be left without operative treatment; this applies especially to those which are produced by bullets, and in which there is no evidence of constitutional disturbance.

In the case of injured men who come under observation only at a later period, when organisms have already invaded 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 and the removal of necrotic tissue: 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 (see p. 14).

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 removed or cut off, and the skin cleansed over an extensive area around the site of injury. Soap and water will doubtless be required, followed by some variety of antiseptic solution; or the new synthetic detergent (cetyl trimethyl ammonium bromide) may be used to clean the skin around the wound.

Owing to the risk of damage to muscle or its vascular supply, a tourniquet should not be used except when haemorrhage is severe.

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.

Surgical incisions aimed at wound excision should be made in the long axis of the limb, and there must be no hesitation about liberally enlarging the skin-wound proximally and distally in order to secure an efficient exposure of the deeper structures. The necessity for vigorous retraction betokens inadequacy of exposure; anything but the gentlest retraction is to be deprecated.

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 muscle, loss of contractility on mechanical stimulation, or failure to bleed, demands wide excision of this most vulnerable tissue. Intermuscular spaces must be opened up, and blood and blood-clot removed, since these constitute an ideal pabulum for bacterial growth. Deep fascia should be freely divided in the long axis of the limb, and even transversely, in order to permit subsequent swelling of the muscles without risk of strangulation of their blood supply.

Fragments of bone which are completely detached should be removed; they act as foreign bodies, and in the event of infection will probably become sequestra. At the same time, great caution must be exercised not to remove too much bone, 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 in which the wound-track actually traverses the bone; 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 likely, unless the skeletal injury is also thoroughly treated. If damaged or doubtful bone requires removal, this should be done by the skilful nibbling or bone-cutting forceps; vigorous tearing at partially detached fragments must never be practised, lest viable and valuable bone be needlessly sacrificed.

No excision of a war wound is complete without the removal of retained foreign bodies, e.g. fragments of high-explosive bombs or shells, clothing, mud, dirt, etc. On the other hand, it is often wiser not to attempt the removal of multiple minute fragments of metal which are buried deeply in the tissues, far from the wound track. If possible, an X-ray film of the injured part should be available at the operation, to aid the surgeon in finding and removing pieces of metal, and to disclose the condition of the bones.

In wound-excision the surgeon must always keep in mind the future function of the anatomical region with which he is dealing: 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 an important muscle, perhaps unnecessarily sacrificing much healthy muscle-tissue.

The preservation of major nerves and blood vessels is of the utmost importance: with adequate exposure these tissues can be thoroughly cleansed. Great care must be taken to preserve the integrity of vessels which remain patent: vascular occlusion may lead to non-infective or infective gangrene. Injury to nerves may cause lifelong disability.

Experiment and clinical experience have indicated that local application of a sulphonamide drug to the recesses of the wound is useful in the prophylaxis of infection (see p. 11). Sulphonamide powders intended for local application in this way must be sterilised, and be applied from sterile containers. The powder should be sprinkled or insufflated over the whole exposed surface after excision has been performed, except that it must not be applied in the immediate neighbourhood of important nerves. By gently massaging the powder into the tissues, any caking will be prevented, and its dissemination facilitated. On no account should the wound be " packed " with the drug, otherwise an undissolved mass will remain as a foreign body. It will seldom be wise to apply more than 15 gm. of sulphanilamide or 10 gm. of sulphathiazole, and the quantities may be proportionately reduced when a sulphonamide has been given by mouth, or microcrystalline sulphathiazole has been instilled earlier. Sulphapyridine and sulphadiazine are less suitable for local application.

In no circumstances should the muscles, fasciae or other deep layers of the wound be sutured. The advisability of primary suture of the skin will depend upon the experience of the surgeon, the interval between the reception of the wound and the time of operation, the site and anatomical characters of the wound, and finally upon the prospect of early transportation. Where the slightest doubt is felt regarding the safety of primary suture, the skin must be left open. Primary suture of battle wounds is generally undesirable.

It is most important that the (vaseline) gauze placed in the depths of the unsutured wound should not be tightly packed: the blood-supply to the structures bounding the wound may be jeopardised by neglect of this instruction. On no account suture the edges of the skin over buried gauze.

The results of closure of a wound within a few days after excision so closely approximate to those of " primary suture," that this method (" delayed primary suture ") is one for consideration, when there is any question as to the advisability of " primary suture."

The presence of a sulphonamide in a wound tends to increase serous discharge and capillary oozing. These should not cause difficulty unless tight suturing has been performed or drainage is otherwise deficient. If suturing is done, sutures should not be drawn too tight and an excess of local sulphonamide must be avoided; in open wounds with adequate drainage larger quantities may be permissible. Plaster-of-Paris may be applied when desired, and the presence of sulphonamide is no contra-indication.

Dressing of Wounds after Operation

In the dressing of wounds after operation, the precautions recommended in *M.R.C. War Memorandum No.* 6 (' The Prevention of " Hospital Infection " of Wounds ')* should be carefully observed.

Sulphonamides have been stated to have an unfavourable effect upon granulation, and so to delay the healing of wounds. It is probable, however, that this will take place only when the amount of the drug introduced into the wounds has been excessive. It is generally unnecessary to apply sulphonamide drugs to open wounds which are healing satisfactorily. On the other hand, the local application of sulphanilamide powder is of particular value in the earliest stages of wound infection by

* H.M. Stationery Office, London, 1941. Price 6*d*. (7*d*. by post).

haemolytic streptococci, to check the spread; it is also valuable in the later stages of healing by granulation, when this is delayed by the presence of streptococci; in such cases, the drug, by inhibiting the streptococci, may actually accelerate, rather than retard, the rate of healing. In septic wounds when sloughs are present, the value of sulphonamides is doubtful, since their efficacy is greatly reduced by the presence of pus and necrotic tissue; when such wounds are being dressed—e.g. after removal of closed plasters—any sloughs, coagulated exudate, etc., should be removed by irrigation with saline, hydrogen peroxide or sodium hypochlorite, before sulphanilamide or, better, sulphathiazole is sprinkled or insufflated over the raw area. It is important to keep the wound moist while the sulphonamide is being applied.

(e) Treatment of Established Gas Gangrene

The most rational method of treating established gas gangrene appears, as in the prophylaxis of the disease, to involve the combined use of surgery, sulphonamide drugs and antitoxin, with the joint objects of controlling the infection and alleviating toxaemia. Once the disease is manifest it increases very rapidly in severity, and the full therapeutic measures should be instituted as soon as the diagnosis is made.

(i) Surgical Treatment

Anaesthesia.—For producing anaesthesia in cases of gas gangrene it is important to avoid the use of toxic agents such as chloroform and ether. The former should never be employed, and the latter only when no alternative drug is available.

The best method is a combination of a small dose of pentothal followed by nitrous oxide and oxygen. The amount of pentothal given will depend on the patient's condition, but the dose must be small and carefully regulated, as patients with gas gangrene are extremely susceptible to this drug, and any attempt to administer a standard dose will be followed by bad results. The oxygen content of the nitrous oxide/oxygen mixture must be kept high and no degree of anoxia permitted. By this technique it will often be possible to obtain satisfactory anaesthesia with a nitrous oxide/oxygen mixture containing 40 to 50 per cent of oxygen.

Surgical technique.—Cases of " anaerobic cellulitis," which may be associated with a foul and gangrenous condition of the superficial tissues, but without involvement of the muscles, are to be distinguished from true gas gangrene affecting muscle. In the former group, toxaemia is often slight, and surgical treatment consists merely in free incision: the ablation of muscular tissue required in true gas gangrene is unnecessary.

The surgical measures demanded in true gas gangrene consist of an uncompromising excision of gangrenous and infected muscular tissue. Where one muscle, or one group of muscles, is involved, that muscle, or muscle-group, must be extirpated from origin to insertion. An ample exposure is necessary, and great care must be taken not to damage the blood supply to adjacent muscles.

Experimental work has shown the important rôle of the lymphatics in the migration of bacteria and the transference of toxins, and that oedema and movement are the chief factors in increasing lymph-flow. The value of " closed " plaster-of-Paris in securing complete immobilization of a limb is incontestable. Nevertheless, facilities for the regular inspection of a wound which has been the site of gas gangrene are desirable, and if plaster is applied, a " window " should be provided. Pain, increase of toxaemia and a worsening of general signs and symptoms are danger-signals that infection is developing, and the plaster should be removed at once. If plaster be used, beware of its being too tight: any interference with the circulation will be disastrous.

In cases of *segmental gangrene*, where the whole limb or a segment of a limb is involved, and in patients with fulminating gangrene, amputation holds out the best hope of saving life. Advice as to the sites and technique of amputation are given in *M.R.C. War Memorandum No.* 5 (' Emergency Amputations ')*. A blood transfusion may be required before amputation is performed. The following special points should be borne in mind in planning amputations for gas gangrene:—

* H.M. Stationery Office, London. 1941. Price 2*d.* (3*d.* by post).

(*a*) The spread of infection in muscle is longitudinal, and spread from muscle group to muscle group occurs only late if at all (p. 6). It may thus be possible at times to leave a stump of useful length by removing individual infected muscle bundles above the point of bone section. A particular example of this is in the leg, where the entire anterior tibial group of muscles may be removed—the posterior tibial group being uninvolved—a useful below-knee stump being left.

(*b*) The presence of subcutaneous crepitation does not necessarily indicate a spread of infection, and flaps may sometimes be fashioned with safety from skin in which crepitation is present. These flaps, of course, should never be sutured.

(*c*) The skin colour changes are no indication of the extent of the subjacent infection (p. 7) and are, in fact, generally less in extent than the infected area.

(*d*) In cases of gas gangrene involving the leg, in which no tibial stump is possible, an amputation through the knee-joint of the Stephen Smith type is quicker and, as no bone section is involved, causes less shock than an amputation through the lower third of the thigh: care must be taken to ensure complete removal of all infected portions of the gastrocnemii. Admittedly, re-amputation will be needed later, but the first duty of the surgeon is to save the patient's life.

(*e*) Guillotine amputations are satisfactory only when immediate after-care with skin-traction is possible. It follows, therefore, that such an amputation should rarely be done as an emergency in the forward area, especially when the patient may have to travel long distances to the base. Flaps of some sort should be fashioned whenever possible, but should not be sutured.

(*f*) In cases of doubt as to the extent of the spread of the infection in the muscles of a limb, it is justifiable to examine the suspected muscles by means of a longitudinal incision before fixing the level of amputation.

(*g*) If a skilled assistant is available, who can control haemorrhage by compression of the main artery, tourniquets are best avoided.

(*h*) Speed is essential to save life.

(ii) Treatment with Antitoxin

In cases of suspected or established gas gangrene every effort should be made to give an adequate dosage of antitoxin *intravenously*, in order to neutralise the toxin as quickly as possible. The dosage of polyvalent gas gangrene antitoxin now recommended for the treatment of gas gangrene is not less than 27,000 units of *Cl. welchii*, antitoxin, 13,500 units of *Cl. septicum* antitoxin, and 9,000 units of *Cl. oedematiens* antitoxin,* injected intravenously, and repeated at intervals of 4 to 6 hours, according to the signs and the response of the patient. Although the risk of anaphylactic reactions is small (see p. 10), a syringe charged with 1: 1000 adrenaline hydrochloride should be kept ready for the treatment of these should they occur. The reaction between toxin and antitoxin is quantitative, so that sufficient antitoxin must be given to neutralise all the toxin formed, if any cumulative effect of unneutralised toxin is to be avoided. Massive doses may be necessary in cases of severe toxaemia but the amount necessary in a particular case can be judged only by the clinical response. A decrease in the patient's pulse rate is generally a reliable sign of diminishing toxaemia.

(iii) Treatment with Sulphonamide Drugs

In cases of incipient or established gas gangrene, sulphathiazole, sulphapyridine, sulphadiazine or, failing these, sulphanilamide should be given in full dosage, as an adjuvant to surgical measures and the administration of antitoxin. The dosage will

* This dosage represents an increase over that recommended in the first edition of this pamphlet. In view of the difficulty of rapidly identifying the infecting Clostridium, it is considered that polyvalent will generally be preferable to monovalent antitoxin for the treatment of gas gangrene.

depend partly on whether the patient has recently received prophylactic administration of a sulphonamide, either by the mouth or locally at operation. When a thorough local application of sulphonamide has been made, half the amount applied locally should be deducted from the total to be given systemically on the same day.

It has been shown experimentally that sulphathiazole, sulphapyridine and sulphadiazine are more active than sulphanilamide against *Cl. welchii* and *Cl. septicum*, and one of the first three drugs should preferably be given in the treatment of gas gangrene. None of the sulphonamide drugs seems to be notably active against *Cl. oedematiens*, and reliance here must be placed rather upon surgical treatment and antitoxin.

The following dosage of whichever sulphonamide drug may be chosen is recommended for the treatment of incipient or established gas gangrene in an adult:—

Initial dose 2 gm. intravenously,* followed immediately by 1.5 gm. by mouth;
1st period (2–3 days): 1·5 gm. 4-hourly by mouth;
2nd period (2 days): 1 gm. 4-hourly by mouth
(approx. ⅔ of dose of 1st period);
3rd period, if necessary (2 days): 1 gm. 6-hourly by mouth.

The duration of the full course will thus be about 7 days, and it should only very rarely be extended. The tablets for oral administration should be crushed and added to 1 oz. of a mixture containing sodium bicarbonate and sodium citrate, 20 grs. of each to 1 fluid oz. The mouth should then be rinsed with water or a glucose drink, since the powder, in acute infections, tends to lodge in the crevices of the mouth. The dosage given should be carefully recorded *in grammes*, and not in terms of tablets, since not all makes of the tablets are of standard 0·5 gm. content.

The dosage of sulphonamides should be controlled, whenever possible, by estimations of the blood concentration of the drug, especially during the first 24 hours of treatment. The concentrations which should generally be obtainable with the respective compounds in the dosage recommended for the acute stage of gas gangrene are as follows:—

	Mgm. per 100 c.c. of blood
Sulphathiazole	6–7
Sulphapyridine	7–10
Sulphadiazine	10–15
Sulphanilamide	7–10

When sulphathiazole, sulphapyridine or sulphadiazine is given, particular care should be taken to ensure that the patient receives a generous allowance of fluids (about 6 pints in 24 hours) to maintain the excretion of a large volume of urine and to minimise the risk of blockage of the urinary passages. A fall in urinary excretion, or the occurrence of lumbar pain or macroscopic haematuria, during treatment with any of these drugs is an indication for immediately stopping the treatment or for changing to the more soluble sulphanilamide. The risk of deposition of the drugs in the kidneys and ureters may be lessened by the administration of alkalies (e.g. the sodium bicarbonate and sodium citrate mixture mentioned above), in sufficient amount to keep the urine alkaline. This is especially important when the bloodstream is suddenly to be flooded with an excess of sulphonamide, though an intravenous injection of sodium sulphathiazole or sodium sulphapyridine. The immediate treatment of symptoms of urinary obstruction includes the application of heat to the loins, and the free administration of fluids and alkalies unless the obstruction is complete. If anuria persists for 12 hours, or if oliguria (less than 500 c.c. of urine per diem) persists for 24 hours, ureteric catheterisation should immediately be performed, and the renal pelves should be irrigated with a warm 2·5 per cent. solution of sodium bicarbonate until the return flow of urine is clear; the catheters should be left in position until urinary function is fully re-established.

* The sodium salts of sulphathiazole or sulphapyridine should be used for the initial intravenous dose. If intravenous injection is impracticable, sodium sulphathiazole or sodium sulphapyridine may be injected intramuscularly, well away from important nerves; some local tissue necrosis will follow the intramuscular injection of these sodium salts, so that intravenous injection is to be preferred whenever possible.

As in the case of all intensive therapy with sulphonamides, the risk of agranulocytosis should be kept continually in mind. Published reports show that, with dosage on the scale here recommended (about 50 gm. in 7 days), that risk is not inconsiderable; and it will be increased if the patient has had a previous course of sulphonamide.

To guard against it, a total leucocyte count *and a percentage polymorph count* should be made, whenever possible, on the third, fifth and eighth days after beginning treatment, and the drugs should very seldom be continued beyond the seventh day. A fall to below 4000 total leucocytes per cmm. or 40 per cent. polymorphonuclears is to be regarded as a serious sign. A watch should also be kept for premonitory symptoms—a rising temperature, headache, lassitude and general ill-being, with (sometimes but not always) a membranous exudate on the fauces or mouth.

In cases where the surgeon has to operate upon a wound in which anaerobic infection is already established, pre-operative administration of a sulphonamide 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 4 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. Alternatively, in patients who are severely ill, sodium sulphathiazole (or sodium sulphapyridine) in doses of 2–3 gm. should be given intravenously (or intramuscularly) half an hour before the operation.

(f) 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. Are the following of value in the treatment of gas gangrene?
   (*a*) Natural antibacterial substances such as penicillin (the restricted supply of penicillin may make it difficult to organise any extensive scheme of clinical tests of its action in the immediate future);
   (*b*) Bacteriostatic agents of the sulphonamide group (the true value of sulphonamide drugs in the prevention and treatment of gas gangrene in man has yet to be assessed);
   (*c*) Antiseptics such as proflavine, 2 : 7-diaminoacridine and 5-aminoacridine, applied locally, either alone or in combination with sulphonamides, etc.
2. What are the clinical characteristics of cases of gas gangrene due to the different causative organisms? Can any correlation be established between clinical features and bacteriology, with a view to appropriate serum or drug treatment? In particular, what are the clinical features of *Cl. septicum* and *Cl. oedematiens* infections?
3. What biochemical changes in the body are associated with the toxaemia of gas gangrene? (Sir Almroth Wright (*Lancet, Lond.*, 1917, **i**, 1) pointed out in the war of 1914–18 that gas gangrene infection produces severe acidosis, but little work has since been done on the subject. It is possible that such acidosis will increase the risk of deposition of crystals of the less soluble sulphonamides in the urinary tract (p. 16) if an adequate fluid intake is not ensured and if alkalies are not given.)
4. What is the most effective treatment for established gas gangrene and for lesser forms of anaerobic wound infection? As mentioned on pp. 3 and 5, Army Form I.1241 and its supplement, I.1241A, 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 with sufficient care and detail to enable the different clinical and bacteriological types of anaerobic wound infection to be differentiated. Exact particulars of the dosage of antitoxin and of drugs used should be recorded, a clear distinction always being made between " prophylactic " and " therapeutic " administration.

III. A 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:—

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

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

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 every case:—

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

Time of Collection of Specimens

The first specimens should be taken before or at the time of operation; they should include *films*, *exudate* and *tissue*. Although no accurate diagnosis can be made from them, Gram-stained films made directly from the wound may indicate to the surgeon the general nature of the infection, and may suggest special methods of culture to the bacteriologist. Specimens of exudate and tissue should be collected at the start of the operation, to establish the nature of the primary infection.

Another specimen should be taken at the time of the first complete dressing. Subsequent specimens should be taken at weekly intervals, to follow the course of the primary infection and to determine the incidence and nature of any secondary or "hospital" infection. The effect of special treatment (e.g. by sulphonamide drugs) should be checked by detailed and frequent bacteriological examinations.

Selection and Collection of Specimens

1. *Films* should be made from muscle at the edge of the affected area, from tissue in the necrotic area, and from exudate from the deeper parts of the wound.
2. *Exudate* should be taken from the deepest part of the wound or from those parts where infection appears to be active. For microscopical work it is most satisfactorily collected in a capillary pipette, but, in general, swabs are more convenient. A suitable swab for both ward and theatre work is one mounted on a wooden applicator 5 inches long. Pipettes and swabs intended for the use of the surgeon during operation must be wholly enclosed inside a sterile receptacle plugged with cotton wool. At the operation, an attendant removes the plug and tips out the end of the swab stick, thus allowing the surgeon to take the specimen and replace it in the tube under aseptic conditions. The attendant then plugs the tube, and labels it for despatch to the laboratory.
3. *Necrotic muscle fragments* from the wound should be placed in a sterile container.

The Nature and Relative Numbers of Bacteria

The primary microscopical examination, and the aerobic and anaerobic cultures, yield information as to the species of bacteria present, and as to their relative numbers, which is of value both to the surgeon and the pathologist. It should, however, always be remembered that a sample may not be representative of the wound flora as a whole.

It must again be emphasised (*see p.* 4) *that the presence of* Cl. welchii *or of other*

toxigenic Clostridia in a wound is not necessarily an indication of gas gangrene. These organisms are often present in the wounds of patients not suffering from that disease.

Procedure

1. *Microscopic examinations of films of material from the wound.*—The films should be Gram-stained. The following points may be useful in judging the nature of the infection.

(*a*) If organisms are present in small numbers but in great variety, with only a few Gram-positive rods, there is probably no serious infection with anaerobic organisms. Very frequently there are large numbers of organisms, in great variety, of both aerobic and anaerobic types. This is especially common when no *débridement* has been performed and there are sloughs present.

(*b*) In a serious anaerobic infection the organisms are more uniform in nature, with a preponderance of Gram-positive rods, and they are generally much more numerous. In infection with *Cl. oedematiens*, however, which is sometimes suggested by the gelatinous nature of the oedema, the organisms may be scanty and are predominantly large rods with a few large oval sub-terminal spores.

(*c*) Thick, square-ended, slightly curved rods of varying length, sometimes Gram-negative or banded, are characteristic of *Cl. welchii*, *Cl. fallax*, and *Cl. bifermentans*; slender rods with oval or round terminal spores may be *Cl. tertium* or *Cl. tetani*, respectively.

(*d*) The presence of boat- or leaf-shaped pleomorphic forms showing irregular staining is almost diagnostic of infection with *Cl. septicum*.

(*e*) Pus cells are seldom numerous even in serious cases of gas gangrene.

2. *Cultural methods.*—The following cultures are recommended:—

(*a*) *Two blood-agar plates*, of unheated exudate and/or muscle fragment, are incubated—one aerobically, or in air plus about 5 per cent. of CO_2 (Appendix, section 5), the other anaerobically in a McIntosh and Fildes' jar. The surface of the anaerobic plate should be well dried before inoculation, to prevent undue spreading of clostridial colonies. An indicator tube should always be used with the anaerobic jar (Appendix, section 6).

(*b*) *Litmus milk*, for the detection of *Cl. welchii* by the characteristic "stormy fermentation." The milk may with advantage contain 0·1 per cent. of agar (Appendix, section 9). The tubes should be long and narrow, and have been boiled to expel any air; on cooling, they should be thickly inoculated from the swab or pus, covered with melted vaseline and incubated without further anaerobic precautions. (*Note:* Many strains of *Cl. welchii* will not give stormy fermentation under the conditions of this test.)

(*c*) *Cooked meat medium.*—This promotes growth of 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 and small numbers of *Strep. pyogenes* and *Staph. aureus* to be detected in subcultures made after some days' incubation. Two tubes are inoculated with unheated material, the first being incubated directly, and the second after heating for 20–30 minutes at 65° C. to kill vegetative organisms. A third tube is inoculated generously with a broth emulsion of exudate or teased-out muscle that has been heated for 5 to 10 minutes in a boiling water bath. The last procedure is a useful method for the isolation of *Cl. oedematiens*. If desired, samples may be taken into additional meat-tubes, varying the period of heating in the water bath at 100° C.; the samples may also be seeded into deep shakes of sugar-free nutrient agar, and into tubes of thioglycollate semi-solid agar (Appendix, section 9), both of which should be examined and plated after 3 and 7 days' incubation (see also Appendix, section 7).

Anaerobic Plate Cultures

These will yield many aerobic organisms, both sporing and non-sporing, 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. *Cl. tertium*, and to a certain extent, *Cl. histolyticum*, will grow aerobically. All anaerobes selected for investigation must be tested aerobically.
3. Colonies of some aerobic spore-bearers, grown anaerobically, often simulate those of Clostridia.
4. The cultures should be examined on the day after inoculation and again after 48 hours, as the colonies of the spore-bearing anaerobes may then be more characteristic.
5. Some anaerobes grow over the surface of agar media in films so fine that only the most careful scrutiny will reveal them.

Spore-bearing Anaerobic Bacteria

There are several published schemes for the identification of Clostridia, some of them devised to give answers rapidly. These schemes are in many ways admirable, and advocate effective methods and tests, but it must be emphasised that the time-consuming difficulties of anaerobic work arise, not so much in identification as in the isolation of pure cultures, especially of delicate organisms mixed with predominant and less fastidious strains. There are no golden rules for the isolation of Clostridia. The procedure outlined above is only a suggestion; each bacteriologist will with experience develop his individual methods.

Though it is desirable to know the full anaerobic flora of a wound, the bacteriologist is concerned chiefly with Clostridia recognised as pathogens, and with the characteristics that distinguish these from the often ill-defined saprophytes of the same genus. Peculiar characteristics are few, and cultural and biochemical tests are often equivocal. The most reliable distinctions are based on pathogenicity, on the specific action of the exotoxins and, in certain cases, upon fermentation reactions. Whenever possible, strains should be tested in animals sensitive to the action of the toxins, and the tests duplicated in animals protected with neutralising antitoxins. The same principle may be applied to *in vitro* tests in sensitive media, using the Nagler reaction for *Cl. welchii* and haemolysis for *Cl. oedematiens* and *Cl. septicum*. The specific protective effect of antitoxin may also be employed in the "filter" method of isolating pathogenic anaerobes from mixed cultures. Thus, if a culture containing *Cl. welchii*, *Cl. septicum* and *Cl. oedematiens* is injected into a mouse passively immunised with *welchii* and *septicum* antitoxins, the proliferation of the first two Clostridia is inhibited, and the *Cl. oedematiens*, producing a fatal infection of the mouse, may be isolated post-mortem.

Immunology of the Toxigenic Gas-Gangrene Clostridia

Bacteria.—None of the pathogenic Clostridia (*welchii*, *septicum*, and *oedematiens*) associated with gas gangrene is antigenically homogeneous. There are at least four serological types of *Cl. septicum*, and a multiplicity of types in the other three species. It is, therefore, impracticable at present to use antibacterial sera for therapy or for the routine identification of strains isolated, though homologous antibacterial sera prepared against the test strains have in certain cases proved effective in the treatment of experimental gas-gangrene.

Toxins.—The toxins of these four Clostridia are species specific, irrespective of the bacterial antigenic structure. The toxins are not single substances but mixtures of various factors, of which the relative proportions vary with each specimen of toxin. Moreover, the antibody response of animals to the toxic components varies, so that antitoxic sera do not necessarily contain similar proportions of the various antitoxins, and indeed may contain no antibody to minor antigenic components in the toxic mixtures. This should be borne in mind when using therapeutic antitoxic sera for diagnostic neutralisation tests. If the factor tested—for example, a haemolysin or a hyaluronidase—is not identical with the lethal factor against which the

therapeutic antiserum is assayed before issue, the antiserum used must be tested for the specific antibody required. The complexity of the toxic filtrates from cultures of these Clostridia is indicated in the following notes on the toxic components, lysins, enzymes, etc., so far described.

Cl. welchii.—The lethality (to mice) of average toxins ranges from 20 to 200 LD50* per c.c. One International Unit of antitoxin neutralises 50 to 100 LD50.

The predominant lethal, haemolytic and necrotising factor (alpha-toxin) is an enzyme (lecithinase) decomposing lecithin. This causes an opalescence in human serum (Nagler reaction), and is neutralised by the antitoxin. The haemolytic action *in vitro* requires ionised calcium, and is inhibited by phosphate, citrate and oxalate. Other components which may be present are a second, oxygen-sensitive, haemolysin activated by sulphydryl groups, e.g. thioglycollic acid; a hyaluronidase (spreading factor) decomposing the mucin hyaluronic acid present in synovial fluid and skin; and a proteinase, attacking collagen, gelatin and other proteins.

Cl. septicum.—The average lethality of the toxin (to mice) is 50 to 200 LD50. per c.c. One International Unit of antitoxin neutralises 25 to 30 LD50.

The lethal toxin causes a liquefactive necrosis in muscle. A haemolysin, proteinases and a hyaluronidase may also be present.

Cl. oedematiens.—The average lethality of the toxin is 1,000 to 10,000 LD50 per c.c. One International Unit of antitoxin neutralises 1,500 to 2,000 LD50.

The lethal toxin causes a gelatinous oedema in muscle. Traces of a lecithinase, and a haemolysin which is not sensitive to oxygen, are usually present.

Cl. histolyticum.—The toxin is relatively weak, but it contains very active proteolytic enzymes, which attack living muscle.

Bacteriology of the Gas-Gangrene Clostridia

The table on pp. 22–23 summarises the more important taxonomic characters of the Clostridia, both pathogenic and non-pathogenic, which occur in wounds. The media listed need not all be used for each strain. Those most useful for moderately detailed routine investigation are blood-agar plates (to show the surface colony form), cooked meat medium, iron litmus milk, iron gelatin, coagulated serum or egg, peptone water (for the indole test), dextrose, lactose, sucrose and salicin. Antitoxin-controlled animal tests provide the most reliable and rapid means of identification of the toxigenic gas-gangrene organisms, and should be made whenever possible.

The following general hints may be useful.

1. Use *large* inocula in Pasteur pipettes from young cultures for fluid media, biochemical tests, etc. 2. See that the plate cultures are not exposed to bright daylight: some strains of Clostridia are photosensitive. 3. Indicators of acid production in " sugar " tubes are liable to irreversible decolorisation by growing anaerobes; hence fermentation tubes showing no colour change after anaerobic incubation should be tested with a drop or two of fresh indicator. 4. Test new procedures with known strains of anaerobes, and for this purpose use other Clostridia (e.g. *oedematiens*) besides *Cl. welchii*, which is one of the least fastidious and most easily manipulable members of the group.

Non-Sporing Anaerobic Bacteria

A small, but definite, proportion of suppurative processes occurring both in civil and military practice is due to non-sporing, usually Gram-negative, anaerobic bacilli. These bacilli include those which have been variously assigned to the *Fusiformis* and (particularly in the U.S.A.) to the *Bacteroides* groups, or have been accorded generic status of their own. Some of the names which have been given to such organisms associated with ulcerative or purulent lesions in man are *Bact. necrophorum* (B. funduliformis), *Bact. pneumosintes* and *Bact. melaninogenicum*; *B. fusiformis, ramosus,* and *nebulosus*; *Bacteroides fragilis, furcosus* and *serpens*. Gram-positive anaerobic

* LD50 signifies the smallest amount of toxin killing 50 per cent. of the animals in the test batch.

TABLE SHOWING CULTURAL AND OTHER CHARACTERISTICS OF SPORE-BEARING ANAEROBIC BACTERIA (1)

Organism	*Surface colonies on horse-blood-agar*	*Deep colonies in nutrient agar*	*Cooked meat medium*	*Alkaline egg*	*Litmus milk*
Cl. welchii (2)	Large, entire-edged, usually haemolytic	Large, lenticular, opaque	Gas, no odour, no digestion, slight pink colour	Opaque, but not clotted	Acid, gas and clot early—stormy fermentation (8)
Cl. oedematiens (novyi)	Clear, slightly irregular, usually haemolytic	Irregular, woolly	Gas, no odour, no digestion	No change	Acid, gas, late coagulation, if any
Cl. septicum (Vibrion septique)	Transparent, spreading, usually haemolytic	Transparent, branching	Gas, no odour, no digestion	No change, or slight opacity	Acid, gas, late coagulation
Cl. chauvoei (3)	Irregular, effuse, transparent, rhizoidal, no definite haemolysis	Irregular, translucent with opaque centre	Gas, rancid odour, no digestion	No change	No change, or occasionally acid and partial clot
Cl. fallax	Large, irregular, opaque	Lenticular, later irregular, opaque	Gas, no odour, no digestion	No change, or slight opacity	Acid, gas, late coagulation
Cl. sordellii (4) } *Cl. bifermentans* }	Irregular, semi-opaque, occasionally haemolytic	Opaque, irregular	Gas, foul odour, digestion, blackening	Opaque, occasionally clotted or curdy	Acid, clot, rapid digestion, later alkaline
Cl. sporogenes (5)	Medusa-head (if plate dry); irregular (if plate moist), haemolytic	Woolly, opaque, with dense centre	Gas, foul odour, digestion, blackening	Opaque, coagulated or curdy	Acid, clot, rapid digestion, later alkaline
Cl. histolyticum	Small, clear, circular or irregular, non-haemolytic. Grows feebly in air	Small, irregularly branching	Foul odour, digestion, blackening, tyrosin crystals (7)	Opaque, coagulated, or curdy	Clot which is rapidly digested
Cl. tetani	Very transparent, spreading, usually haemolytic	Delicate, filamentous	Slight foul odour and digestion, slight blackening	No change, or opaque, but not clotted	No change or occasional coagulation
Cl. tetanomorphum	Small, circular	Small, irregular	Gas, no odour, no digestion	No change	No change
Cl. cochlearium (6)	Entire-edged, delicately crenated, slightly granular, non-haemolytic	Lenticular, later loosely filamentous	No change	No change	No change
Cl. tertium	Small, clear, entire-edged, non-haemolytic; some strains grow well in air	Small, lenticular	Gas, no odour, no digestion	Slight opacity	Acid, gas, late coagulation

Organism	*Iron litmus milk* (9)	*Iron gelatin* (10)	*Coagulated serum or egg*	*Indole*	*Dextrose*	*Lactose*	*Sucrose*	*Salicin*	*Spores*	*Pathogenicity* (12)
Cl. welchii (2)	Acid, clot and marked gas early, no digestion, no blackening	+ No blackening	–	–	AG	AG	AG	– or AG	Seldom seen in culture	Usually + to mice and guinea-pigs
Cl. oedematiens (novyi)	Acid, gas, late if any coagulation, no digestion, no blackening	+ Occasional blackening	–	–	AG	–	–	–	Central and subterminal, very few	+ to mice and guinea-pigs
Cl. septicum (Vibrion septique)	Acid, gas, late coagulation, no digestion, no blackening	+ No blackening	–	–	AG	AG	–	AG	Central or subterminal	+ to mice and guinea-pigs

Cl. chauvoei (3)	Acid, gas, late if any coagulation, no digestion, no blackening	+	–	–	AG	AG	AG	–	Central or sub-terminal	+ to guinea-pigs, occasionally + to mice
Cl. fallax	Acid, gas, late coagulation, no digestion, no blackening	–	–	–	AG	AG	AG	AG	Seldom seen in culture	When freshly isolated + to mice and guinea-pigs
Cl. sordellii (4) *Cl. bifermentans*	Acid, gas, rapid digestion, odour, blackening	+ Blackening	Disintegration or digestion	+	AG	–	–	– or AG	Central or sub-terminal	+ to guinea-pigs –
Cl. sporogenes (5)	Acid, gas, rapid digestion, odour, blackening	+ Blackening	Digestion	– Vanillin (11) violet +	AG	–	–	– or AG	Central or sub-terminal	–
Cl. histolyticum	Gas, rapid digestion, odour, blackening	+ Red or orange	Digestion	–	A or –	–	–	–	Central or sub-terminal	Usually + to mice and guinea-pigs
Cl. tetani	No change, or occasional coagulation	+ Blackening	– or slightly softened	+	–	–	–	–	Terminal, round	+ to mice and guinea-pigs
Cl. tetanomorphum	No change	–	– or slightly softened	–	AG	AG	–	–	Terminal round	–
Cl. cochlearium (6)	No change	– Occasional blackening	–	–	–	–	–	–	Terminal, oval	–
Cl. tertium	Acid, gas, late coagulation, no digestion, no blackening	– Blackening	–	–	AG	AG	AG	AG	Terminal, oval; formed only anaerobically	–

NOTES TO TABLE

(1) Motility has not been included in the table. With the exception of *Cl. welchii* and possibly *Cl. fallax*, all the anaerobes mentioned in the table and the subjoined notes are motile. A positive result from a motility test is of greater value than a negative, since the motility of flagellated *Clostridia* is often difficult to establish. For the best results, motility should be tested in as nearly as possible anaerobic conditions; a portion of a young, rapidly growing anaerobic broth culture is sealed into a length of capillary tubing, leaving a minimum of air space at the ends, and examined immediately under a $\frac{1}{6}$-inch objective.

(2) Among the saccharolytic, non-proteolytic Clostridia, *Cl. butyricum* and *Cl. multifermentans* may be confused with pathogenic species. In many respects *Cl. butyricum* resembles *Cl. welchii*, but it ferments salicin as well as sucrose, and fails to liquefy gelatin. Since salicin-fermenting *Cl. welchii* strains occur, the safest distinguishing features are non-liquefaction of gelatin and the antitoxin-controlled test in mice or guinea-pigs. *Cl. multifermentans* closely resembles *Cl. butyricum*, except that it ferments mannitol. Toxigenic *Cl. welchii* is readily identified by the Nagler reaction (see Appendix, section 11).

(3) *Cl. chauvoei*, characteristically the cause of blackleg in cattle, is included for the sake of completeness. It has been described by German workers as occurring also in war wounds, but the strains may have been confused with *Cl. septicum*. It has never been so reported by British workers.

(4) The distinction between *Cl. sordellii* and *Cl. bifermentans* rests entirely upon pathogenicity. The two should probably be treated as a single species, with varying toxigenic powers.

(5) There are some proteolytic Clostridia (*parasporogenes*, *centrosporogenes*, *aerofoetidum*, etc.) which differ only slightly from *Cl. sporogenes* and *Cl. bifermentans* in their biochemical and cultural reactions. Since these organisms occur only as secondary invaders in human infections and are non-toxigenic, there is little to be gained in further distingnishing them. Some of the strains produce smooth entire-edged colonies.

(6) There is a group of Clostridia with terminal or almost terminal spores which, like *Cl. cochlearium*, fail to ferment any sugar, and might thus, on biochemical grounds, be confused with *Cl. tetani*. Tests in mice will establish the identity of, at any rate, the toxigenic strains of *Cl. tetani*. *Cl. tetanomorphum*, morphologically indistinguishable from *Cl. tetani*, is non-toxigenic, does not liquefy gelatin and ferments dextrose.

(7) It is the relatively early deposition of crystals in cooked meat medium which characterises *Cl. histolyticum*. White nodules and crystals are deposited in older cooked meat cultures of other Clostridia, especially *Cl. bifermentans*.

(8) This appearance may be simulated by other microbes, and especially by mixtures of aerobes and anaerobes.

(9) See Appendix, section 10.

(10) + indicates liquefaction: see Appendix, section 10.

(11) Tested for indole by the acid vanillin method, peptone water cultures of *Cl. sporogenes* turn violet when exposed to air for a few hours. None of the other species listed does so.

(12) The guinea-pig is generally the most suitable experimental animal for pathogenicity tests of anaerobes, but other animals may be substituted; e.g. for *Cl. welchii*, the pigeon and mouse, and for *Cl. septicum* and *Cl. oedematiens*, the rabbit, pigeon and mouse.

diphtheroid bacilli have been found in septic wounds. The incidence and significance of all these bacteria in war wounds needs investigation.

Anaerobic 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. Some of these are true anaerobes; others will not grow aerobically when first isolated, but 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. Strains may best be maintained for future study in cooked meat medium. It is suggested that a microscopical examination of the original meat culture should be made after some 3 days' incubation, and if streptococci are seen which did not appear in the culture plates, further cultures should be made on blood-agar anaerobically, and streptococcal colonies subcultured into " sloppy " dextrose-agar (Appendix, section 9). Other anaerobic cocci, having staphylococcal, diplococcal or tetrad arrangements may be found, but nothing is known of their importance.

Aerobic Plate Cultures

It should be borne in mind that, besides the anaerobes detailed above, a large variety of pathogenic and saprophytic organisms can establish themselves in wounds, and may be of the utmost clinical significance. They include *Streptococcus pyogenes*, the pneumococcus, *Strep. faecalis*; anaerobic streptococci and streptococci of the viridans type; *Staphylococcus aureus*, *Staph. albus*, other Gram-positive cocci and Gram-negative cocci; *Bact. coli*, *Bact. alkaligenes*, *Bact. lactis aerogenes*, other coliform and paracolon bacilli, *Proteus vulgaris* and *Ps. pyocyanea*; aerobic spore-bearers; *C. diphtheriae*; a large variety of diphtheroid bacilli, including those resembling actinomyces; *Haemophilus influenzae* and *H. parainfluenzae*. The commonest aerobic microbes infecting wounds are *Staph. aureus* (coagulase positive), *Strep. pyogenes* and coliform bacilli, in that order of frequency.

Staphylococci should be tested for coagulase, irrespective of any presumptive identification based on pigment formation (Appendix, section 3).

Haemolytic streptococci should be tested for soluble haemolysin (Appendix, section 2). If this is present, either in aerobic or anaerobic culture, the strain should be grouped, and if necessary, typed (for Reference Laboratory, see p. 25).

REFERENCE LABORATORIES

If, in any laboratory, it is impossible to proceed with the isolation or identification of anaerobes, a meat tube should be inoculated with the wound material or unidentified culture, *sealed*, and sent to a Reference Laboratory. The following are addresses to which cultures of anaerobes may be sent for identification in the British Isles:—

Professor A. Fleming, M.B., F.R.C.S., F.R.S.,
Inoculation Department,
St. Mary's Hospital,
London, W.2.

Professor J. McIntosh, M.D., LL.D.,
Central Laboratory, E.M.S. Sector 5,
Stoke Mandeville Hospital,
Bucks.

Professor A. A. Miles, F.R.C.P.,
Central Laboratory, E.M.S. Sector 4,
Shrodells,
Vicarage Road,
Watford, Herts.

Professor J. W. McLeod, M.B., F.R.S.,
The School of Medicine,
Leeds, 2.

V. D. Allison, M.D., D.P.H.,
Emergency Public Health Laboratory,
Institute of Preventive Medicine,
The Parade,
Cardiff.

Professor C. H. Browning, M.D., L.L.D, F.R.S.,
Pathology Department,
The University,
Glasgow, W.2.

Professor J. Cruickshank, M.D.,
Department of Bacteriology,
Foresterhill,
Aberdeen.

Professor T. J. Mackie, C.B.E., M.D., F.R.S.E.,
Bacteriology Department,
University New Buildings,
Teviot Place,
Edinburgh.

Professor W. J. Tulloch, O.B.E., M.D.,
Department of Bacteriology,
Medical School,
60 Small's Wynd,
Dundee.

N. C. Graham, M.C., M.B., D.P.H.,
Department of Bacteriology,
Institute of Pathology,
Grosvenor Road,
Belfast.

Streptococci known to produce soluble haemolysin may be sent for grouping and typing to:—

S. D. Elliott, M.D., D.P.H.,
Research Laboratory for Streptococcal Infections,
Queen Charlotte's Hospital,
Ravenscourt Square, London, W.6.
(*Telephone No.*: Riverside 2174)

APPENDIX

1. Neutralisation of Sulphonamide Activity in Pathological Materials.

Specimens taken from wounds treated with a solid sulphonamide may contain large quantities of it, especially if the drug is relatively insoluble. Usually the spreading of the material on a plate, or its inoculation into broth, inactivates the drug by dilution. If sulphonamide inhibition in culture is suspected, the specimen should be suspended first in sterile broth or saline containing 0·05 per cent. *p*-aminobenzoic acid. A 0·5 per cent. solution of the acid in saline may be autoclaved as stock, and one volume added to nine of the broth or saline. The inhibiting action of *p*-aminobenzoic acid is confined to the sulphonamide drugs.

2. Detection of Soluble Streptococcal Haemolysin.

The streptococcus is grown in 15 per cent. serum broth for *not more than* 15 hours (e.g. overnight). Equal volumes of the culture and 5 per cent. washed horse red blood 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 after 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.

3. Coagulase Test for Staphylococci.

Seed the coccal colony into nutrient broth containing human or rabbit plasma. The plasma concentration required is one to two drops (about 0·05 c.c.) to each c.c. of broth. The size of tube for the test should be selected so that the volume of broth chosen forms a fluid column of a height at least twice its diameter (e.g. for 1 c.c. of broth, use a 75 × 8 mm. tube). On incubation of the culture at 37° C., an obvious coagulum appears in one to six hours with the majority of potentially pathogenic and toxigenic *Staph. aureus* strains: after 18 hours almost all such strains produce a coagulum.

Alternatively, a 1: 10 dilution of the plasma in saline may be used; this gives a lower proportion of positive results than the plasma broth unless the inoculum is either large (e.g. 5 well-grown colonies) or consists of a young turbid broth culture (6 hours old) in the logarithmic growth phase, 0·25 c.c. being added to each c.c. of plasma saline. Unfiltered citrated or oxalated plasma is required; the clear fluid above a discarded bottle of stored blood works admirably, but each batch must be tested for full activity with a strain of known *Staph. aureus*. The plasma keeps well at 2° C.

4. Selective Bacteriostatic Methods in Solid Culture Media.

Bacteriostatic agents may either be incorporated in the agar or spread over the already inoculated plate. In the latter method, 2 to 3 drops (60 to 100 c.mm.) of an appropriate concentration are spread over half the plate, so that the inhibited and the uninhibited growth from the same culture may be directly compared.

(*a*) Gentian violet, in blood-agar, 1: 300,000, inhibits staphylococci, micrococci, Gram-positive and Gram-negative bacilli, and permits the growth of haemolytic streptococci. For surface application, use 1: 3,000. A batch of dye of known efficacy must be obtained.

(*b*) Potassium tellurite in nutrient agar inhibits haemophilic bacilli and most coliforms in a concentration of 1 in 500,000, and *Ps. pyocyanea* in a concentration of 1 in 50,000. The reaction of *Proteus vulgaris* is variable; most strains are resistant to 1 in 50,000. Streptococci, staphylococci and diphtheroid bacilli are resistant to 1 in 10,000. The action of the salt on anaerobes is selective, but the effective concentrations for different species have yet to be determined. The salt is most useful for the inhibition of coliforms, a concentration of 1: 50,000 being used for incorporation in the medium and 1: 1,000 for surface application.

(*c*) Mechanical bacteriostasis can be applied to prevent overgrowth by *Proteus vulgaris*. A blood agar plate is inoculated in the usual way, and then covered with a thin (1–2 mm.) layer of melted agar at 45° C. Colonies of *Proteus vulgaris* growing between the two layers of agar do not spread, and other types of colonies may easily be picked out from among them. Some Proteus usually spreads round the edge of the agar on to the upper surface. The spread can be prevented by flooding the upper agar surface with 2–3 c.c. of methylated spirit, leaving for 15–30 seconds and draining off excess spirit. The plate is dried at 37° C., tilted agar surface downwards with the lid off, and then incubated in the usual way.

5. Influence of CO_2 on the Growth of Bacteria.

Certain streptococci, pneumococci, haemophilic bacteria, actinomyces and other organisms grow badly or not at all unless there is from 2–10 per cent. of CO_2 in the air. If possible, the effect of an atmosphere of air containing 2–10 per cent. of CO_2 on the growth of anaerobes and aerobes from war wounds should be observed. A simple apparatus for this purpose consists of a cylindrical tin container, about 6" diam. by 8" high, with a " press-down " lid. The cultures, either plates or tubes, are inserted, and if a CO_2 supply is available from Kipp's apparatus or a cylinder, sufficient gas is run in to make the CO_2 content about 5 per cent. A wide margin of error (2–10 per cent.) in CO_2 concentration is permissible. The lid is then pressed on without further sealing. If, however, a constant CO_2 supply is not available, an open tube containing excess of HCl may be inserted and a marble chip dropped into it before the lid is pressed down. A concentration of approximately 5 per cent. CO_2 will be given by a marble chip weighing about

(0·25 V) grams in (2·5 V) c.c. of 25 per cent. HCl, where V is the volume of the container in litres. The increased pressure in the container is unimportant. Further cultures may be added without adding fresh marble, provided that this is done quickly.

6. Indicators and Dryers for Anaerobic Jars.

(*a*) An indicator of anaerobic conditions is made by adding 2 drops of methylene blue staining solution to 2 per cent. dextrose broth which has been brought to a pH of 8–8·5 by the addition of 2 drops of *N*/10 NaOH. The fluid should become colourless in the jar after 2–3 hours at 37° C., and should remain so if anaerobic conditions are maintained. The indicator may be used repeatedly if the growth of contaminants in the fluid is inhibited by the addition of 0·01 per cent. merthiolate or phenyl mercuric nitrate, or of a little thymol.

(*b*) Excessive spreading of Clostridial colonies on agar surfaces may be prevented by keeping the atmosphere in the jar relatively free from moisture. Anhydrous granular $CaCl_2$ in a perforated container or a petri dish lid serves the purpose well.

7. Deep Agar Cultures for the Isolation of Anaerobes.

If anaerobic jars cannot be obtained or devised, the isolation of Clostridia may be accomplished in deep agar shake cultures. Approximately tenfold serial dilutions of the exudate or extract of teased-out tissue, or of cooked meat culture of a specimen, are made in broth. The carry-over of 0·5 c.c. into 5 c.c. lots of broth is sufficiently accurate for this purpose. Usually, 4–5 dilutions suffice, but an examination of a stained smear of the material will indicate the number to be made. The undiluted material, and the dilutions, are mixed with nutrient agar at 45° C., which is then allowed to set. After incubation, it will be found that one of the dilutions of material yields colonies sufficiently discrete to display deep colony characters of the various anaerobes, and to allow their transfer to other media by Pasteur pipette after dividing the tube with sterile precautions. Care must be taken to avoid the fine spreading films of bacterial growth that sometimes develop between agar and glass. Since the sampling of deep colonies often necessitates a transverse division of the tube, a saving of test-tubes may be effected by drawing the inoculated agar into plugged pasteur pipettes made of 5–7 mm. tubing, having a wide portion at least 20 cm. long. The agar is drawn to within 0·5 cm. of the plug, leaving a short air space at the tapering end, which is then sealed. The sealed tip is wiped with strong antiseptic and the tubes are incubated in the horizontal position.

8. Negative Staining for the Detection of Clostridial Spores in Culture.

The sample of culture is mixed on a slide with a drop of 10 per cent. nigrosin (containing formalin as a preservative); it is spread out into a thin film by means of a wire and examined after drying. In the thicker parts of the film the spores stand out as clear spots, while the bacillary portions are partly overlaid by the nigrosin. Alternatively, a bacterial film may be made on the slide; when it 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; confirmation of the existence of spores can be obtained by direct staining methods.

9. Media for Growing Anaerobes in the Presence of Air.

Many fluid media, provided that they are heated immediately before use, can be made to support the growth of anaerobes incubated in the presence of air, by the addition of small quantities of agar (0·1–0·2 per cent.; " sloppy " agar media), and of reducing substances. Both should be used if possible, but if reducing substances are not available, the agar alone is helpful, whether the medium is to be incubated in air or in anaerobic apparatus (e.g. in litmus milk for the detection of stormy fermentation). Some of the reducing substances are dextrose (1–2 per cent.), ascorbic acid (0·1 per cent.), thioglycollic acid (Brewer's medium), and reduced iron (see below).

Nutrient broth containing 0·1–0·2 per cent, agar and 0·02 per cent. thioglycollic (thiolacetic) acid, adjusted to pH 7·4 with NaOH immediately before autoclaving, is an excellent medium. Tubed medium keeps for weeks at room temperature; it should be boiled and cooled immediately before use. It may be enriched with dextrose or any other carbohydrate. A useful " sloppy agar " is quickly made by the addition of 0·5 c.c. of molten nutrient agar to 5 c.c. of warm broth.

Ordinary laboratory broth and peptone-water media may be readily converted by the addition of iron strips into media that permit the vigorous growth of anaerobes in air. Heat the tubes of media in boiling water for 10 minutes, and cool them quickly without shaking; to each tube add a 3 × 25 mm. sterile strip of mild steel sheeting (" sheet-iron " about gauge No. 26) and inoculate in the usual way. In this medium, sugar reactions and indole tests may be elicited after 1–2 days' incubation, from Clostridia, anaerobic streptococci, and *Fusiformis* and *Bacteroides* strains. Early growth is sometimes difficult to detect, since the medium becomes clouded with iron hydroxides, but with large inocula, the most fastidious anaerobes can safely be assumed to have grown in iron-media. (For the characteristic action of Clostridia on iron-milk and iron-gelatin, see below.)

10. Iron-Milk and Iron-Gelatin Media for Anaerobes.

Iron-milk and iron-gelatin are useful additions to test media for anaerobes (Spray). The iron may be added as non-rusty filings, as strips of black stove-pipe iron sheeting, or even as fragments of iron nails. Milk is thereby converted to a medium in which Clostridia grow in the presence of air, and in which the production of gas and of blackening are added to the range of possible

changes displayed by milk media. The reactions are summarised in the Table (pp. 22–23). Spray lists five types of reaction:—I. Active gaseous fermentation, clot in 12–48 hours, violently disrupted by gas; no digestion nor blackening—*Cl. welchii, butyricum, aerofoetidum* and *multifermentans*; II. Inactive gaseous fermentation, coagulation in 4–6 days; no digestion nor blackening—*Cl. sphenoides, fallax, tertium* and *septicum*; III. Long-continued inactive gaseous fermentations, coagulation (if any) in 10–30 days, no digestion nor blackening—*Cl. oedematiens* and *chauvoei*; IV. Inactive gaseous fermentation; rapid digestion with or without clotting; strong blackening—*Cl. sporogenes, bifermentans-sordellii, histolyticum*; V. No action—*Cl. tetani, tetanomorphum, cochlearium*. "Inactive gaseous fermentation" is characterised by a fine stream of bubbles rising from the bottom of the tube. In group II the gas production is usually more obvious than in groups III and IV, since the gas is trapped in the relatively quickly formed clots.

In iron gelatin (incubated at 37° C.), blackening is produced by certain strains, and *Cl. histolyticum* produces a wine-red colour; liquefaction occurs as in plain gelatin. The iron should be kept in dry sterile tubes and added immediately before inoculation; stored iron media tend to discolour.

11. Rapid Detection of Cl. welchii in Direct Plate Culture (Nagler Reaction).

The following medium permits a serologically controlled identification of toxigenic *Cl. welchii* within 20 hours of plating a wound swab.

Mix 2·65 c.c. of human serum with 0·65 c.c. of Fildes' extract (peptic digest of sheep's blood), warm to 50° C., and add 10 c.c. of molten nutrient agar at 50° C. (i.e. 20 per cent. serum and 5 per cent. extract). Pour the plate, dry for 2 hours open in the incubator, and mark into halves. On one half, spread 2–3 drops (about 0·1 c.c., 50–100 international units) of *Cl. welchii* antitoxin, and allow to dry. Inoculate both halves of the plate with the swab, spreading the inoculum in the same pattern on each side. On the test half, 18–20 hour *Cl. welchii* colonies are surrounded by a zone of opacity due to the Nagler reaction, while morphologically similar colonies on the antitoxin control side produce no change in the medium. The reaction depends on the power of the major component of *Cl. welchii* exotoxin (the alpha toxin) to split a soluble lipo-protein complex in human serum, with the formation of an insoluble precipitate of lipoids and proteins. The reaction is specifically inhibited by *Cl. welchii* antitoxin.

The 20 per cent. serum may be added to any medium which encourages both growth and toxin-formation by *Cl. welchii*, provided that it is clear enough to permit the detection of the Nagler opacity. If desired, a separate control plate of human serum agar may be used, containing about 4 units of antitoxin to the c.c. A batch of human serum should be selected which gives a good reaction with a known toxigenic strain of *Cl. welchii*; it will keep its full Nagler reacting properties for several months at 2° C.

Some streptococci, and a few aerobic and anaerobic spore-bearers, also give zones of opacity, the formation of which is at most only partially inhibited by antitoxin. *Cl. oedematiens* is among these; if polyvalent gas-gangrene antitoxin is used for the control cultures, in place of monovalent *Cl. welchii* antitoxin, it must be remembered that the feeble *oedematiens* zones may also be specifically neutralised on the control agar. *Cl. sordellii* and *bifermentans* give zones specifically neutralised by *Cl. welchii* antitoxin, but these reactions are usually feeble. These two organisms can be readily distinguished from *Cl. welchii* by the presence of spores in stained smears of the 24-hour colonies, and, if necessary, by their inability to ferment lactose.

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