NATIONAL HEALTH INSURANCE

MEDICAL RESEARCH COMMITTEE

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Report on the Anaerobic Infections of Wounds and the Bacteriological and Serological Problems arising therefrom

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NATIONAL HEALTH INSURANCE

MEDICAL RESEARCH COMMITTEE

REPORTS

OF THE

COMMITTEE UPON ANAEROBIC BACTERIA AND INFECTIONS

REPORT ON THE ANAEROBIC INFECTIONS OF WOUNDS AND THE BACTERIOLOGICAL AND SEROLOGICAL PROBLEMS ARISING THEREFROM

Medical Research Committee

(National Health Insurance.)

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15, BUCKINGHAM STREET,
STRAND, W.C. 2.

The Committee upon Anaerobic Bacteria and Infections.

WITH a view to the better co-ordination of inquiries into the characters of anaerobic organisms, with special reference to the bacteriology of anaerobic wound infections, the Medical Research Committee invited the following to serve as a special investigation Committee for this purpose:

Professor WILLIAM BULLOCH, M.D., F.R.S. (*Chairman*).
W. E. BULLOCK, M.D.
S. R. DOUGLAS, M.R.C.S., L.R.C.P. (Captain I.M.S. retd.).
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Miss MURIEL ROBERTSON, M.A. (*Secretary*).
C. G. L. WOLF, M.D.

(*with corresponding members*).

INTRODUCTION

Among the wound complications which confronted all the belligerents in 1914, none was more serious than ' gas gangrene ', whether considered from the point of view of incidence or of mortality. Although this remarkable condition was known and described in pre-antiseptic days, it had not been a disease of any consequence in any of the wars of the twentieth century. The outbreak of the Great War, however, brought it early into prominence, and it was at once recognized as a toxic-infective condition. The unusually high incidence on the Western Front, where fighting took place upon a long cultivated soil contaminated with human and animal excrement, led to the belief that the infective agents were soil bacteria, and it was early established in all the countries interested that these micro-organisms were of the anaerobic type. In spite of the accumulation of much knowledge on the subject of anaerobes, a study of bacteriological literature before the war shows clearly that the descriptions published by different writers were for the most part widely divergent, and agreed in only a few instances. From the frequency with which *Bacillus welchii* (*B. perfringens*) was found, apparently in pure culture, it was considered that this was the chief aetiological agent, and it was actually named ' the bacillus of gas gangrene '.

A careful scrutiny of the discharges from wounds by more refined methods during 1916 and 1917 revealed the fact, however, that gas gangrene could not be regarded as an aetiological entity, but could be caused by a series of pathogenic anaerobes acting singly or in combination with each other or in association with certain well defined non-pathogenic anaerobes. By degrees Pasteur's *Vibrion septique* was disentangled from the mass of anaerobes, and a number of other anaerobes, new to science, were discovered, among which may be specially mentioned the highly toxic *B. oedematiens*. The isolation of these bacteria was chiefly the work of MM. Weinberg and Séguin of the Pasteur Institute in Paris, whose conclusions were confirmed and extended by English bacteriologists, notably Major McNee, Captain Adrian Stokes, Captain Herbert Henry, Captain W. E. Bullock (now Gye), with the Forces Overseas, Dr. James McIntosh, working at the London Hospital in the service of the Medical Research Committee, and Miss Muriel Robertson of the Lister Institute of Preventive Medicine. Major W. J. Tulloch in the laboratories of the Royal Army Medical College and the Lister Institute made at the same time unusually successful researches into the bacteriology of tetanus. Captain C. G. L. Wolf, working for the Medical Research Committee, by the application of exact methods added important contributions to the biochemistry of anaerobes, first at Boulogne and later at Cambridge. In America, Major C. G. Bull and Dr. J. L. Stoddard added considerably to our knowledge of the bacteriology of anaerobes.

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The advances which were made were the result of applying the most refined and most difficult forms of bacteriological technique, and they depended also upon the early recognition of the fact that many, if not most, anaerobes have an inveterate tendency to live in close association with other anaerobes. This is an association which can only be recognized by the most careful and prolonged scrutiny of what appear to be pure cultures, under diversified and often complicated conditions. To the failure to realize this must be ascribed the fact that in the bacteriological literature of the Central Powers the knowledge of gas gangrene was and still remains (1919) in a state of hopeless confusion, and it cannot be doubted that it has been the researches of French and English bacteriologists that have cleared away the mystery of the aetiological agents producing gas gangrene. The outcome of this advance in knowledge led the French to test at an early date the prophylactic and curative effects of the serum of animals immunized with pure cultures of specific pathogenic anaerobes. Among the most successful workers in this field must again be mentioned Weinberg and Séguin, and Major C. G. Bull. They were the first to apply the serum treatment in man. In England the earliest attempts to produce sera were those of McIntosh and O'Brien, the latter of whom, in association with Captain H. Henry and Captain W. E. Bullock, ultimately undertook, with the assistance of the Committee, the preparation of sera in the Wellcome Research Laboratories on a large scale for the British Army. Throughout the course of these inquiries the British workers were greatly aided by French bacteriologists and surgeons, among whom may be specially mentioned Weinberg, Vaucher, Duval, and Chutro. It is a pleasant duty for the Medical Research Committee to record their thanks for the help received from those French workers who with unusual generosity placed their unpublished records in the hands of the British, and kept us in close touch with all the developments in their experience of gas gangrene.

This wide co-operative effort towards gaining new knowledge and applying it to the production of curative sera was chiefly focussed in this country in the work of the Committee upon Anaerobes which the Medical Research Committee had appointed in March 1917, in the hopes of bringing more closely together the various workers engaged. The constitution of this special committee is given at the head of this Report. In the autumn of 1918 it seemed certain that if the war continued there would be available large supplies of a highly potent serum, both for preventive and curative use, in which the defence against the three chief organisms already mentioned was to be combined. Happily the armistice intervened to remove the urgent need for this, and the practical demonstration of the fruits of these long investigations must be expected now only in the infinitely rarer occasions of peace.

It appeared to the Medical Research Committee that every effort should be made to gather up and clarify the scientific results which had so far been obtained. The prolonged study of gas gangrene in all the chief countries had led, moreover, to the production of many writings, both good and bad, and this gave further reason for the preparation of an authoritative and critical report upon the various

parts of the subject by workers with first-hand knowledge. The special Committee upon Anaerobes were invited accordingly to complete their labours by preparing the Report which is now presented.

At first it was intended to deal completely with the aetiological, experimental, and sero-therapeutic aspects of the gas gangrene problem, and work on these lines had proceeded to a considerable extent, when in 1918 appeared the admirable, exhaustive monograph, entitled *La Gangrène Gazeuse,* by MM. Weinberg and Séguin. This necessitated a revision of the plans of the Committee, who deemed it inadvisable merely to traverse the same ground as the French writers. It was considered best to concentrate especially on a critical analysis of the experiences of the individual members of the Committee, and to undertake, where necessary, fresh investigations to clear up the doubtful points and to utilize the practical results gained by the use of the British-made sera in the treatment of gas gangrene.

The Report as it stands is the conjoint work of the members of the Committee. For the sake of completeness, however, several sections were written by others who had special knowledge of certain aspects of the anaerobic and even aerobic microbic complications of wounds. Thus the Committee received a report on 'the clinical features and treatment of anaerobic infection of wounds and gas gangrene' by Dr. John Fraser, of Edinburgh, who had an almost unique experience of the disease while serving in France. Dr. Alexander Fleming prepared a summary of his extensive researches on the 'aerobic infections of wounds', while Major W. J. Tulloch gave the benefit of his wide experience on the bacteriology of tetanus. Dr. E. H. Kettle, of St. Mary's Hospital, undertook for the first time an exact inquiry into the finer pathological anatomical changes in human and experimental gas gangrene, which is published in the Appendix. Professor W. Bulloch compiled an extensive bibliography of the literature on anaerobic infections, and personally examined almost all the papers for the purposes of this report, as a help to future investigators who may not have been in touch with much of the medical war literature.

The Medical Research Committee believe that they are speaking for a wide circle of workers when they express their own cordial thanks to the members of the Anaerobe Committee, not only for their arduous individual work in the laboratory, but even more for the time and effort they have given so ungrudgingly to share their knowledge with others and to contribute in discussion to the common object in view. Thanks are due especially to Professor William Bulloch, who from the beginning has acted as chairman of the Committee, and to Miss Muriel Robertson, who by kind permission of the governing body of the Lister Institute was able to undertake the difficult duties of secretary, which brought a heavy burden of correspondence with many workers at home and abroad.

The work of the special Committee was necessarily done in close touch with the Army Medical Service, and here the Committee would offer their grateful acknowledgements to the Director-General, A.M.S., and to many other officers, for the encouragement and direct

assistance which made it possible to link effectively the work done in civilian laboratories at home with that done in the armies and with the practical problems offered by Service needs and conditions. To the Army Medical Department the Committee are indebted for the supply of many data from Medical Case Sheets, which have been important for the study of the sero-therapy of gas gangrene in the field.

To the editors and publishers of the *Journal of Pathology and Bacteriology* the Committee are indebted for permission to reproduce a large number of the illustrations given in the plates. Other illustrations are taken from the report by Dr. James McIntosh, previously published by the Committee (*Special Report Series*, No. 12). Figures 76, 77 and 78 are reproduced from the beautiful drawings made by Mr. Thornton Shiels from preparations by Dr. McIntosh, and the Committee believe that these are perhaps the most exact representations yet published of the infective conditions displayed.

Medical Research Committee
15 Buckingham Street,
Strand, W.C.2.

September 1919.

REPORT ON THE ANAEROBIC INFECTIONS OF WOUNDS AND THE BACTERIOLOGICAL AND SEROLOGICAL PROBLEMS ARISING THEREFROM

CONTENTS

INTRODUCTION

The serious consequences which may result from the multiplication of anaerobic bacteria in wounds have furnished a medical problem in the present war which, both because of its gravity and of its novelty, has been studied by clinicians and bacteriologists of most of the belligerent nations.

The following pages summarize the present state of knowledge of wound anaerobes, of the clinical conditions which they may cause, and of experience in the preparation and the use of antisera.

The advances chronicled are due to many investigators, who, by means of improvements in technique which have made it easier to obtain pure cultures, have studied the large amount of material which the war has provided.

I. THE CLINICAL FEATURES AND TREATMENT OF ANAEROBIC INFECTION OF WOUNDS AND GAS GANGRENE

By John Fraser, M.C., M.D., F.R.C.S. Ed.

Anaerobic infection with its sequel of gas gangrene has been a common and an extremely serious complication of infected war wounds.

When the present war began our knowledge of the subject was limited. The condition had been noted as occurring in previous campaigns, and on rare occasions it had been met with in civil practice, but we possessed little trustworthy information regarding the pathology and the treatment of the condition. It has been through the medium of expensive and painful experience that the knowledge which we now possess has been gained.

In the subsequent remarks the anaerobic infection of wounds is dealt with from the point of view of clinical features and treatment.

1. General Clinical Considerations.

(i) *The Type of Wound.*

There are certain types of wound which are especially liable to anaerobic infection and these are wounds which illustrate one or both of two conditions, imperfect drainage and extensive devitalization and death of tissue with extravasation of blood.

The first mentioned condition is typically illustrated in the deeply penetrating wounds with a small point of entrance, widespread destruction of the more friable muscles and possibly retention of the infected projectile at the base of the wound. Such wounds are usually produced by fragments of bombs and grenades, by shrapnel bullets, and by small fragments of H.E. shell. The projectile is generally travelling at a comparatively low velocity and hence the tendency for the missile to be retained.

The second type of wound associated with tissue death in the part affected is usually caused by irregular fragments of H.E. shell. These fragments are generally travelling at such high velocity that there is widespread tissue destruction not only in the course of the missile but also in a considerable zone of tissue outside the actual track.

(ii) *The General Condition of the Wounded Man.*

It is certain that the general condition of the wounded man has an important bearing on the development of the infection. It has been observed that gas gangrene is more prevalent among fatigued troops, who have undergone long spells in the trenches, than it is among fresh soldiers who have gone direct into action. In a similar way, men who have suffered from severe haemorrhage and shock subsequent to the wound are specially liable to the development of this complication.

(iii) *The Nature of the Infecting Agent.*

It is essentially true that the more irregular the projectile fragment, the more likely is anaerobic infection to follow. There are certain definite reasons for this statement. (1) An irregular fragment of shell produces widespread devitalization and tissue destruction. This is well seen when such a wound is studied in comparison with that produced by a rifle bullet. A short range rifle bullet on the other hand may produce as much destruction as a large irregular shell fragment. (2) The more irregular the projectile fragment the more likely is it to carry into the wound pieces of infected clothing. (3) As the majority of H.E. shells burst on percussion with the ground, irregular fragments carry with them into the body of the wounded man portions of soil which in highly fertilized districts may be heavily charged with anaerobes.

(iv) *The Vascular Supply.*

A factor of extreme importance is the question of the blood supply to the part. A diminished supply of blood to the wounded area predisposes the part to the development of an anaerobic infection. A variety of causes may lead to the interference with the vascularity: the severe haemorrhage which is so often the concomitant of war wounds results in a general anaemia: the nature of the wound is often such that a tourniquet has been applied and the distal blood supply has thus been arrested: in a more local sense the contusion and tissue destruction which has resulted from the wound leads to a thrombosis in the blood vessels for some extent around the wound. At any rate, in whatever way it may have been caused, an impoverished blood supply to the part is a potent factor in favouring the development of an anaerobic infection.

(v) *The Regions and Tissues Affected.*

Anaerobic infection spreads with great rapidity in muscle and more slowly in areolar tissues. It is interesting to observe that all

muscles are not equally liable to the infection; for example, it is comparatively rarely that one finds any widespread infection of the muscles of the back. The larger and coarser-fibred muscles, e.g. the glutaei appear to be the most liable to the infection. It is possible that this distinction really depends upon the blood supply. In scalp wounds uncomplicated by compound fractures of the skull and in wounds of the face, gas gangrene is a rare complication.

The serous cavities of the body would appear to offer considerable resistance to anaerobic infection. The disease in these situations progresses as a rule more slowly and is naturally limited in extent to the region of the infected membrane. Although the infection of a serous membrane is thus for anatomical reasons a localized infection, yet the absorption of toxine from such a focus may yield a progressive intoxication which either by itself or in conjunction with an ensuing anaerobic septicaemia is fatal to the individual. This is particularly true of infection of the pleura.

(vi) *The Question of Interrupted Drainage from the Wound.*

Any factor which interferes with efficient drainage from the wound predisposes to the anaerobic infection. This fact may be illustrated by the type of wound. The deep narrow punctured wound is obviously liable to the infection. A slit-like skin wound may contract, effectively blocking the escape of discharge and at a deeper level there may be considerable retention of infected material. In the same way a foreign body may prevent efficient drainage, and in the early days of the war there were numerous instances in which packing of the wound or insertion of antiseptic pastes acted in a similar way with disastrous results.

(vii) *The Clinical History of the Wound.*

The period of time which elapses before the wound shows signs of infection varies over a wide range. Changes which indicate an anaerobic infection may be apparent within an hour of the injury being sustained; on the other hand, several days may elapse before distinctive features appear. The changes in the wound which are associated with the infection may be summarized as follows.

There is a scanty foul-smelling discharge composed of broken-down blood clot; it is of a brownish colour, and mixed with it there are bubbles of gas. The odour of the discharge appears to depend on the type of infecting organism, and on the state of the infection: in the early stages a characteristic smell is absent, later it becomes foul and acrid. The skin around the wound assumes a faint purplish colour, and there is a local swelling due to the accumulation of brown serous fluid in the subcutaneous tissues. There is a similar infiltration throughout the intermuscular septa and connective tissue planes. Throughout the precincts of the wound there is an infiltration of gas and it extends to further limits along certain lines—the subcutaneous tissues, the perivascular tissues, and the planes of intermuscular connective tissue.

Up to this point in the clinical history of the wound, the muscle is not infected. When it does become infected, the nomenclature

must be altered ; it is no longer an anaerobic infection of the wound but the condition of gas gangrene. The muscle is infected in one of two possible ways :

1. The muscle (usually an injured one) is gradually invaded by the organisms, which starting from the wound make their way towards the extremities of the muscle until it is totally destroyed. The blood supply of the muscle is intact. In such cases, the line of invasion may be seen and distinct zones of infection recognized. At the actual wound, the muscle is black, friable, and diffluent. Next comes a zone in which the predominant colour is red. Separating the red zone from the healthy muscle is a yellow band, irregular in outline, somewhat raised above the surface and hard to the palpating finger. In the majority of cases, however, no such differentiation into zones is apparent. In these a loss of contractility is the first thing to be observed, and when this has been established the muscle rapidly changes its colour through varying stages of dirty red and greenish yellow to a dark diffluent mass.

2. The muscle may undergo what is really a post-mortem infection, for it has already died in consequence of the blood supply having been cut off. In such a case the appearance of the muscle varies from a purplish red to a greenish black diffluent mass.

Soon after the muscle has been infected, gas can be demonstrated in it, at first as bubbles between the muscle fibres, afterwards in the surrounding areolar tissues. The gas has a deleterious effect in so far as it exerts pressure within the fascial sheath of the limb, constricting the blood-vessels and producing that amount of interference with the blood supply which is so favourable to an extension of the infection.

2. Varieties of the Disease.

Clinically, several types of the disease may be recognized.

1. Localized anaerobic infection in the wound.
2. Slowly spreading anaerobic infection in the wound.
3. Gas gangrene of the ' group ' type when a single muscle or a group of muscles is attacked.
4. Gas gangrene of the ' massive ' type where a whole segment of a limb is involved.
5. The fulminating type.

3. Symptoms and Signs of Gas Gangrene.

(i) *Symptoms.*

The features depend on whether it is early or late. In the early stages, distinctive symptoms are absent but pain is generally marked. The unusual amount of pain probably depends upon increasing pressure within the wound.

When the infection becomes established and begins to spread, distinctive signs arise. The pain increases and there is a feeling of numbness in the superficial parts of the limb. Constitutional symptoms begin to make their appearance ; the patient looks distressed and ill ; the lips acquire a cyanotic colour ; the pulse rate is rapid and the temperature rises by three or four degrees.

Vomiting is frequently present. If the disease continues to extend, the constitutional symptoms become more marked. The pulse becomes more rapid and is finally running and uncountable; vomiting becomes more frequent, the extremities are cold and blue and the temperature falls. It is a remarkable fact that the mind generally remains acute even to the end. In the terminal stages some degree of general icterus may be present.

Death frequently occurs with such dramatic suddenness as to suggest the occurrence of pulmonary embolism, although it is to be noted that this has never been found at autopsy.

(ii) *Physical Signs.*

The physical signs differ according to the type of disease which is present. If there is a localized anaerobic infection of the wound, the only distinctive sign may be a foul-smelling discharge mixed with bubbles of gas. As the disease extends, swelling of the part is noted and percussion of the swollen area yields a tympanitic note.

(iii) *The Group Type.*

In the ' Group ' type the limb may show no departure from the normal or it may be swollen to a greater or less extent. The skin may be normal in appearance or it may be tense or blanched from the underlying swelling. As a rule, the area around the wound is tympanitic and crepitation may be detected. In a further stage the swelling of the limb increases, the skin acquires a dusky hue and tympanitis and crepitation are more marked. At a still later period, the skin shows mottling with purple patches and finally becomes a greenish yellow. It must be borne in mind that the gangrene of the muscles may be far advanced though covered by a skin which is normal in appearance. In certain cases the skin may show the development of large irregular bullae filled with a blood-stained serous fluid.

If the muscle is exposed, it presents at first a dry brown appearance changing to a dirty red and, finally, it becomes pultaceous, and black in colour with a slimy surface.

(iv) *The Massive Type.*

This type occurs generally in a segment of a limb from which the blood supply has been cut off by the interruption of the main vessel. There are two groups of features which are met with in this variety of the disease.

1. The limb which is already in a condition of dry gangrene from the interruption of its blood supply suddenly becomes swollen and tympanitic and the patient exhibits the constitutional symptoms, pain, vomiting, rise of temperature, and rapid pulse.

2. The gangrene, the tympanitis, and the constitutional features manifest themselves at the same time.

In both varieties, during the early stages the appearance of the skin is that seen in ordinary arterial gangrene, but infection by bacteria causes a more rapid appearance of the signs of decomposition.

(v) *The Fulminating Type.*

The fulminating type stands out in contrast to all the other varieties. Here it would appear that either the individual powers of resistance are low or the infection is very virulent. The type may appear as a primary manifestation, more commonly it occurs as a secondary feature of the 'Group' or 'Massive' type. It is associated with severe pain, extensive swelling of the affected part, rapid spread of the disease, and severe constitutional features.

4. Complications of Gas Gangrene

Apart from the local spread of the infection and, the gas gangrene toxaemia which is invariably present, there are two complications which may occur:

1. Gas gangrene septicaemia.
2. Gas gangrene pyaemia (metastatic gas gangrene).

Gas Gangrene Septicaemia. Under certain conditions invasion of the blood-stream with the organisms of gas gangrene occurs; such an invasion produces the features of gas gangrene septicaemia. It is interesting that the general signs of the condition are by no means so acute as those of gas gangrene toxaemia, but in contrast to the latter, the general appearance is good, there is a swinging temperature, normal in the morning and 102 or 103 in the evening; there is progressive wasting and this in spite of the fact that the appetite may remain good. In the majority of cases pyogenic organisms eventually gain entrance to the blood-stream and to this super-added infection the patient rapidly succumbs.

Gas Gangrene Pyaemia. This complication is rare, but a number of instances of its occurrence have been recorded. It generally follows a gas gangrene infection associated with a compound fracture. There is an invasion of the blood-stream and secondary deposits of gas gangrene organisms occur throughout the body; crepitant swellings containing gas appear in the muscles and subcutaneous tissues. These secondary foci of gas gangrene often develop in various parts of the body where the tissues have suffered some slight damage as, from the introduction of a hypodermic injection or an infusion of saline, or they may develop in tissues subjected to prolonged pressure as in the buttock when the patient lies in bed tilted on one hip. The prognosis is extremely bad.

5. The Diagnosis of Gas Gangrene.

There are certain conditions which may be mistaken for gas gangrene. These are:

(*a*) Extensive haemorrhage into the tissues, generally from a wound of a large blood-vessel.

(*b*) Post-traumatic oedema of the tissues.

In the case of haemorrhage, the limb while swollen is firm to the touch, dull to percussion and there is an entire absence of the constitutional features which are so typical of gas gangrene infection. The situation of the wound may give an indication of the source of the haemorrhage; blood clot is generally escaping from the

wound and X-ray examination demonstrates the absence of gas in the tissues. Pain while present is generally much less severe than in gas gangrene. If the swelling is due to tissue oedema, there is acute pain on palpation, a dull note on percussion, herniation of the underlying tissues through the wound, and absence of constitutional symptoms.

The diagnostic features of a gas gangrene infection are: pain, crepitation, resonance on percussion, and, above all, severe constitutional symptoms.

6. The Treatment of Gas Gangrene.

(i) *Preventive Treatment.*

There are certain conditions which undoubtedly favour the development of gas gangrene. They may be summarized as follows:

(*a*) Retention of extravasated blood.
(*b*) Interference with the local circulation.
(*c*) The presence of masses of devitalized tissues.
(*d*) Extensive fractures and comminution of long bones.
(*e*) Retention of wound secretions by dressings, pastes, or packing.
(*f*) Delay in the mechanical cleansing of the wound.
(*g*) Retention of foreign bodies.

It is, therefore, evident that steps should be taken to avoid any of these predisposing factors. It is of the utmost importance that any constricting influence on the limb should be avoided.

Cases in which there is any suspicion of gas gangrene occurring should be evacuated to some centre where efficient surgical treatment can be carried out as rapidly as possible.

(ii) *Treatment by the Administration of Alkalies.*

All patients suffering from this disease should be given alkali by the mouth. If there is vomiting and the general symptoms are marked, the alkali (4 per cent. sodium bicarbonate in normal saline) should be given intravenously—fifteen to twenty ounces are generally sufficient.

(iii) *Surgical Treatment.*

The surgical treatment will necessarily be guided by the general condition of the patient and the extent of the gangrene. Thus a patient who is in good condition can be subjected to an operation for extirpation of the diseased muscles while another patient with the same amount of disease, but whose general condition is bad, will require to be treated by amputation.

The surgical measures to be adopted are best considered according to whether the condition is:

(1) A localized infection.
(2) Group gangrene.
(3) Massive gangrene.
(4) Fulminating gangrene.

(1) *Localized Wound Infection.* The most efficient treatment of this condition is complete removal *en bloc* of the affected part. After primary excision of the wound with skin and fascia, and after prolonging the incision to expose the deeper structures, an attempt is made to remove the whole of the injured and infected tissue *en masse* together with any foreign material. This leaves a large fresh wound surface which though almost invariably infected is at least easier to sterilize than the original wound. Excision, *en bloc*, is often impossible on account of the involvement of such structures as bone, large arteries, or nerves. In such instances dead muscle, and other soft structures which are obviously a source of danger are cut away, after all injured and ragged skin has been cleanly excised; infected tissues which cannot be removed are carefully cleansed. The wound is then immediately subjected to a process of continuous sterilization by the Carrel-Dakin method. It is important that the wound should not be closed in any way immediately after operation, even though complete excision has apparently been carried out. The question of secondary suture can be considered when repeated bacteriological examinations of the part show that the proper degree of sterility has been secured. The operative treatment outlined must be carried out with all possible speed—a long operation is most harmful.

(2) *Group Gangrene.* In this type of the disease the treatment will vary according to whether the general condition of the patient is good or bad.

(*a*) *Patient in good condition.* The mechanical cleansing of the wound is carried out by the method which has already been described. The affected tissues are exposed by adequate longitudinal incisions. The condition of the muscles is carefully observed. All muscular tissue is removed which does not contract or bleed when cut into, or which shows any departure from the normal colour.

Certain muscles may require to be removed *in toto*, that is from origin to insertion; in this way a limb may be preserved which, judging from the degree of crepitation and tympanitis present, would otherwise have been sacrificed. After excision has been performed, a Carrel-Dakin dressing is applied.

(*b*) *Patient in bad condition.* This is most likely to occur in fractures of the long bones. In these cases, amputation at the site of fracture is generally indicated. If on account of the crepitation and tympanitis of the limb, it is thought that a higher amputation is necessary, it is advisable to investigate the condition of the muscles through skin incisions which are made at the level of the injury: in this way it can be ascertained whether the muscles are dead at the level of the proposed removal.

When gas gangrene affects the tissues of the leg it is seldom justifiable to amputate through the thigh; in these cases, a guillotine amputation is performed through the head of the tibia or through the knee-joint, the heads of the gastrocnemius muscles being then removed from their attachment to the femur. By this procedure the operative shock is lessened, a painful stump is avoided and an increase of length of limb is gained.

(3) *Massive Gangrene.* As this type is usually the sequel to injury of the main vessel of the limb, every attempt should be made in cases of wounds of the larger blood-vessels to maintain the circulation until at least such a time as the collateral circulation has been established. This may be done by arteriorrhaphy or by the use of Tuffier's tubes.

When gangrene has occurred and the limb is obviously dead, amputation is the only possible method of treatment. As regards the level at which the amputation should be done, the condition of the muscles may be accepted as a guide and the limb should be removed at the lowest level of the living muscle.

In cases in which there is grave injury such as a shattered joint or a fracture of a long bone, it may be necessary to disregard the extent of the gangrene and amputate at a higher level as dictated by the injury. In massive as in group gangrene, amputation through the knee-joint or head of the tibia is to be preferred to amputation through the thigh.

(4) *Fulminating gas gangrene.* Here the rate of spread of infection is so rapid that within a few hours not only may the whole limb be gangrenous but the process may have extended for some distance on to the trunk. Immediate amputation by the most rapid means is the only treatment which offers any possibility of success. The disease may have extended to such a level that the amputation must be performed through tissues which are already infected. This need not be taken as a contraindication to operation, for if the flaps are stitched back and continuous sterilization by the Carrel-Dakin method is carried out, the progress of the disease becomes arrested in a certain proportion of cases.

This type of the disease often appears after amputation through the thigh; in these cases re-amputation at or near the hip is almost uniformly fatal and should only be undertaken with caution.

In cases in which the disease has spread from the limb to the trunk, high amputation should be carried out, and the infected area above the amputation should be treated by free incision.

(iv) *Methods of Amputation in Gas Gangrene.*

Whenever possible, short flaps of some description should be made; the wound is kept open for several days and is irrigated by the Carrel-Dakin method. Below the knee, where amputation will almost always be of a provisional nature, the guillotine method is often the best. It may be performed through the head of the tibia or through the knee-joint and the cut surface of the amputation stump should be carefully examined. It should be borne in mind that the lesion is not a horizontal one, but owing to certain muscles being infected and others not, the upper level of the disease is very uneven, mounting much higher at one point than another. For this reason the appearance on section of an individual muscle may afford evidence that it is infected, the disease having extended locally above the general level. If such be the case, the individual muscle should be dissected out. In this way the disease is eradicated and length of limb saved.

(v) *General and Post-operative Treatment.*

The usual remedies for the treatment of shock are carried out. Stimulants are given freely with advantage. As all cases are suffering from some degree of acidosis, it is important to administer an alkali. It may be given by mouth (sodium bicarbonate 8 drachms to one pint of water) or if the general condition is poor and there is persistent vomiting, it may be given intravenously (4 per cent. solution of sodium bicarbonate). The serum treatment, both prophylactic and therapeutic, is under trial and as yet no definite statement can be made as to its efficiency.

II. INCIDENCE OF GAS GANGRENE.

During the different phases of the war the incidence of gas gangrene among the wounded varied greatly. The highest figures were always observed during active operations, when the wounded were not collected with the usual rapidity and when treatment was, on that account, delayed. Again, the incidence varied according to the site of the battle area, being most frequent in highly cultivated soils. The figures given below must be looked upon as approximate rather than accurate on account of the difficulty in collecting the necessary information.

The highest incidence of gas gangrene undoubtedly occurred in the early period of the war about the time of the first battle of the Marne and the heavy fighting in the Ypres salient. No actual figures have as yet appeared, but surgeons in charge of casualty clearing stations in these areas were of opinion that among the wounded the incidence was well over 12 per cent. From that date the condition of affairs improved very markedly in the British army. In the spring and summer of 1918, during heavy fighting, the figure was only about 1 per cent. In the first and second armies, among 13,303 wounded, there were 158 cases (1·1 per cent.) of gas gangrene in the forward stations, while for the same period in the base hospitals, out of 23,792 wounded, there were 92 cases of gas gangrene (0·39 per cent.).

The figures given by Bowlby (1919) are very similar in that out of a total of 25,060 patients at certain base hospitals during 1917 and 1918 there were only 84 patients (0·33 per cent.) with severe or ' massive ' gas gangrene, while at one base during the great retreat of March 1918 the incidence of gas gangrene among 20,000 wounded was 1·0 per cent.

Many factors contributed to this reduction, chief amongst which may be mentioned, more efficient surgical cleansing, earlier evacuation of wounded, and prophylactic measures.

In the French armies the incidence appears to have been rather higher. Ivens (1916) had 107 cases of gas gangrene among 1,694 wounded (6·3 per cent.), while Chalier and Chalier (1) give the figure as 5·4 per cent., and Ombrédanne as 13 per cent. (cited by Guermonprez).

For the German army on the Western front, Wederhake puts the incidence at 2·2 per cent. and Franz at 2 per cent. Rumpel's figures are more detailed: in one war hospital, from May to September 1916, among 3,036 wounded there were 114 cases of gas gangrene,

an incidence of 3·7 per cent. He also gives statistics for one of the armies from the middle of December 1916 until the end of March 1917; there were 5,921 wounded with 170 cases of gas gangrene, a total incidence of 2·8 per cent., but during an offensive the incidence rose to 7 per cent. By January 1917, however, following upon more energetic surgical treatment and the employment of anti-gas gangrene serum, the incidence was reduced to 0·6 per cent.

On the Eastern front, on the other hand, the general incidence was lower, as might have been expected. Out of 5,000 wounded, Wieting observed gas gangrene in 1·43 per cent. of the cases.

Although the morbidity from gas gangrene was considerably reduced amongst all the belligerents, nevertheless even an incidence of 1 per cent. constituted a serious loss on account of the high mortality (20–50 per cent.).

III. BACTERIOLOGY.

Introduction.

A perusal of the pre-war literature of anaerobic bacteria reveals the state of disorder which prevailed in this branch of bacteriology. First, in the matter of technique there was no method of choice by which surface growths could be easily obtained; many methods were detailed but none was entirely satisfactory. Further, it cannot be doubted that most of the cultures of the anaerobes described were impure, and consequently the descriptions were inaccurate. This is seen in the case of such a well-known and important organism as *B. oedematis maligni*, which is described by von Hibler and later by Felix von Werdt (1912) as proteolytic. It is highly probable that *B. enteriditis sporogenes*, Klein, represented a mixture of *B. welchii* and *B. sporogenes*. Such errors were numerous, were copied from book to book, and led to sterile controversy which ended in general confusion.

No attempt will be made in this Report to clear up the confusion except where absolutely necessary, nor to put names to the numerous organisms insufficiently described in the older books and journals.

The errors were due for the most part to the fact that the problems of anaerobic infections were of academic interest and were not thoroughly studied; and in part to the very great inherent difficulties of the subject.

The war transformed these problems into questions of high importance and urgency; the sufferings of our men and the grievous losses of our armies compelled professional bacteriologists to attack the subject with renewed vigour and enlisted to their aid colleagues from kindred branches of biological science. The united efforts of all these workers, with their different disciplined experiences, have advanced knowledge, enlarged our comprehension of wound infections, and aided in abating suffering.

The advances in knowledge may be thus summarized:—

It may be said that it is now not much more difficult to obtain surface cultures of such anaerobes as *B. tetani* or *vibrion septique* than of common aerobes. This fundamental advance is due to improved methods of anaerobic cultivation, among which should

be especially noted the beautiful adaptation by McIntosh and Fildes of Laidlaw's method of obtaining anaerobiosis by the use of finely divided platinum or palladium as a catalyst.

The pathogenic and probably most of the non-pathogenic anaerobes which may infect wounds contaminated with soil, have been isolated and described. The list of these microbes is long, but the majority are apparently non-pathogenic. The principal pathogenic species are *B. welchii, vibrion septique*, and *B. oedematiens*.

These three organisms, acting alone or in combination, are responsible for almost all of the acute rapidly-evolving cases of gas gangrene where the whole course of the infection may be run in the space of 8 to 48 hours. They may be helped in their pathogenic activity by the so-called non-pathogenic anaerobes and also by aerobes, but our knowledge on this point is not precise. It is certain, however, that they all assist one another.

B. welchii is responsible for a larger percentage of cases of gas gangrene than either of the other two, though it is now known that the percentage is not so high as it was formerly thought to be. *Vibrion septique* is commoner than *B. oedematiens*.

The rôle played by the more numerous but less dangerous non-pathogenic anaerobes has not been clearly defined; they may be responsible for some of the features of gangrenous wounds—such as putrefactive odour, blackening and digestion of tissue—both in the acute cases and in local anaerobic infections. Certain strains of *B. histolyticus* undoubtedly play a large part in some cases of gas gangrene (Weinberg).

It should be noted that any one, or possibly any combination, of these organisms may be found in a wound which is not, and does not become, gangrenous. This point will be discussed in the section devoted to experimental gangrene.

The part played by *B. tetani* will be dealt with separately. Finally, in some cases, specific names are used in this Report for species which are believed to comprise several varieties.

The anaerobes infecting wounds may, therefore, for practical purposes, be considered under the following headings.

1. Pathogenic anaerobes liable to set up conditions of gas gangrene with symptoms of generalized intoxication.
2. *B. tetani.*
3. Wound infecting anaerobes whose action may be ancillary to the causal agents of gangrene and tetanus.

1. Pathogenic Anaerobes liable to set up Conditions of Gas Gangrene with Symptoms of General Intoxication.

(i) *B. welchii.* Migula, 1900. (Synonyms: Bacillus of Achalme, 1891; *B. aerogenes capsulatus*, Welch and Nuttall, 1892; *B. phlegmonis emphysematosae*, Eug. Fraenkel, 1893; *B. perfringens*, Veillon and Zuber, 1898; B. *enteritidis sporogenes*, Klein, 1895).

B. welchii is a non-motile organism, uniformly Gram-positive in young cultures; in old cultures Gram-negative individuals are frequently met with.

Morphology. The length of the bacilli varies from 4 to 8 μ, and the breadth from 1 to 1·5 μ. In general they are straight and the ends are usually rather square.

Very short, almost coccal forms, and long filaments may be found under certain conditions of growth. Some strains show curved rods.

Involution forms showing considerable 'pleomorphism' (club shapes, filaments, tadpole forms, granular types, &c.) are found, particularly in old cultures upon coagulated serum.

A capsule may be demonstrated; it is most conspicuous in rods from the exudate of infected tissues or from media containing serum, but it does not appear to be absent at any time (M. Robertson).

The organism has no flagella.

Spores. Sporing forms of *B. welchii* are sometimes to be found in infected wounds, but are most readily formed in such media as casein broth, alkaline egg broth, and coagulated serum, all of which are rich in protein and free from fermentable carbohydrates. They are rarely seen in media containing substances which the bacillus is able to ferment. Individual strains of the organism vary a good deal in the readiness with which they form spores even in the most favourable media. The spores are relatively large, oval in shape and with slightly flattened ends. They are subterminal or central in position; the body of the rod in which they develop disintegrates very rapidly.

Anaerobiosis. *B. welchii* does not require very strict anaerobic conditions for its growth.

Colony. Surface colonies on nutrient agar, serum agar, or glucose agar are circular in contour. In 24–48 hours they may attain a size of 1 to 2 mm. in diameter; older cultures may become much larger, the edges of the colonies sometimes being crenated. Young colonies (12 hours) are translucent; they soon become opaque and granular. There is a variety of the colonies of *B. welchii* which shows a very thick opaque centre and a granular slightly crenated margin. The latter are of a viscid consistency and tend to adhere to the medium In deep agar shake cultures the colonies are lenticular.

Cultural reactions.

Meat medium:	Very rapid growth, with the development of a pinkish colour; gas; and an acid reaction. The meat is not blackened, and there are no macroscopic signs of digestion. The odour is sour but not putrefactive.
Milk medium:	Vigorous reaction; rapid acid clot with evolution of much gas, i.e. so-called 'stormy fermentation'. This reaction takes place as a rule within 12 to 48 hours; spores are not formed.
Coagulated serum:	No change; no liquefaction. Spores are usually formed in this medium; filaments, granular forms, and many involution types may be seen, especially in old cultures.
Alkaline egg broth:	The medium becomes evenly opaque but is not precipitated in large flocculi.
Gelatine: [1]	Liquefied.

[1] Throughout this Report the gelatine reaction is recorded upon the result obtained by incubating the inoculated tube at 37°C. for 48 hours, and thereafter cooling it by immersion in a beaker of cold water.

Cultural reactions (continued).

Broth : Active growth ; medium becomes turbid and opaque ; there is evolution of gas ; after a period of time varying between about 24 to 48 hours the organisms sink to the bottom of the tube. The reaction becomes strongly acid.

Substances fermented.	Substances not fermented.
Glycerine (variable)	Mannite
Glucose	Dulcite
Laevulose	Salicin.
Galactose	
Maltose	
Saccharose	
Lactose	
Inulin (variable)	
Starch.	

Animal reactions.—The pathogenicity of the various strains differs considerably, many being non-pathogenic, but under constant conditions the virulence of a given strain does not, as a rule, show much alteration. Other strains, however, which have been propagated upon artificial media for long periods of time may show considerable loss of virulence.

The lethal dose of different strains of *B. welchii* in broth culture is variable within fairly wide limits for pigeons, guinea-pigs, and mice ; rabbits show a considerable resistance, but they can be infected if massive doses are used.

Washed bacilli suspended in saline are non-pathogenic. Spores of *B. welchii*, when washed off serum slopes with saline, fail as a rule to produce any effect whatsoever in the animal, though slight local induration of the limb injected may occur.

If a lethal dose (0·1 to 1 c.c. according to the virulence and condition of the culture) of a broth culture is injected into the muscles of the thigh of a guinea-pig, an extensive oedema is produced which involves the limb injected and which may spread over the whole abdomen and into the axillary region. The animal usually dies within 24 to 48 hours, but spontaneous recoveries do occur even with doses of a pathogenic broth culture which are lethal for the majority of animals of the same weight. Injection in a guinea-pig may produce a perforating necrosis of the skin and subcutaneous tissue, in which case recovery not infrequently takes place. Sub-lethal doses produce lesions of greater or less severity from which the animal recovers.

A post-mortem examination reveals an extensive oedema, usually slightly blood-stained, but never to the same extent as with infection by *vibrion septique*. There may be some gas in the tissues ; the muscles of the injected limb are of a pale pink colour. They are very friable and soft and give the appearance of being digested. The odour is sour but not putrefactive.

The internal organs in guinea-pigs do not show any conspicuous macroscopic alterations, except the suprarenal glands, which may be of a deep red colour, especially where the death of the animal

has taken place rapidly. In other cases they may be mottled or hardly altered at all. If the animal survives until the third day, the suprarenals do not as a rule show discoloration.

In the case of mice the liver is small and pale in colour, the kidneys and suprarenals are congested, and the bladder is sometimes full of blood-stained fluid. The duodenum is constantly dilated and often pink in colour from congestion of its vessels.

In all cases the blood-stream is invaded at a relatively early stage of the infection.

Microscopic examination. Very large numbers of bacilli are to be found in the muscles at the site of inoculation, and these may also be numerous in the exudate. Spores are not formed in the body of animals experimentally infected with cultures of *B. welchii*. Chains of 3 to 5 elements may be seen on the peritoneal surfaces. Long filaments are never observed.

Toxin. A soluble toxin can be demonstrated in young (24 to 48-hour) broth cultures; the nature and action of this toxin is discussed in another section.

Agglutination. Agglutinins for *B. welchii* cannot be demonstrated in the blood of rabbits which have been repeatedly inoculated intravenously with the organisms. Weinberg claims that horses which receive massive intravenous injections of *B. welchii* emulsions over a long period of time do sometimes develop agglutinins for the homologous strain, but the reaction is usually restricted to this and cannot be applied for diagnosis.

As a general rule a good emulsion can be made in saline, although certain strains show a tendency to spontaneous agglutination.

Distribution in nature. *B. welchii* is a very frequent and widely spread organism. It is present in the intestine of men and animals. It is found in many samples of milk, in earth and dust, and can be cultivated from clothing and practically from any object exposed to dust.

(ii) *Vibrion septique.* Pasteur et Joubert (1) (1877). *Synonym B. oedematis maligni.* Koch.

This microbe has been the centre of much controversy; it has, however, become clear that an organism agreeing in characters with Pasteur's *vibrion septique* is of frequent occurrence in wounds. Numerous strains have been isolated and the characters of the bacillus are now perfectly well known. *Vibrion septique* and *B. oedematis maligni* of Koch are probably identical. Many writers subsequent to Koch, such as von Hibler, C. O. Jensen, von Werdt, and others, have, however, repeatedly described *B. oedematis maligni* as liquefying serum and digesting meat with the production of a putrid odour. These reactions do not obtain in pure cultures of undoubted *vibrion septique*, the inference being that these writers and even certain quite recent workers, such as Conradi and Bieling, were dealing with impure cultures (see p. 93).

Confusion has also arisen in the tendency to consider *vibrion septique* as identical with *B. chauvoei* (bacillus of Rauschbrand). This is undoubtedly an error. *B. chauvoei* is quite distinct from *vibrion*

septique, but strains of *vibrion septique* have been isolated from cases which appeared clinically to be symptomatic anthrax and also from accidental wounds in animals. The most recent discussion of this subject will be found in the paper of K. F. Meyer (1915).

Morphology. Vibrion septique is a Gram-positive organism; it is motile in young cultures and in the exudate from infected animals. It presents a rather wide range of different forms according to the conditions of culture. In broth or in meat medium the organisms appear as rods of varying length somewhat more slender than *B. welchii*. Spores are readily formed and are usually situated towards one extremity; central spores are, however, not uncommon. Deeply stained bulb-like types may be present, especially in young cultures. In fluid media containing fresh tissue and on coagulated serum very varied appearances may be seen, such as 'navicular' or 'citron' types, i. e. pale, citron or boat-shaped bodies with deeper staining points at one or both extremities, deeply staining club-shaped forms, filaments, and bulb-like types often growing in short chains. The navicular forms may be observed in films made directly from infected tissues and blister fluid, &c.

Cultural Reactions. Colony. Vibrion septique is a strict anaerobe. Surface colonies can be obtained on plates under good conditions of anaerobiosis or upon the surface of serum agar slopes. Serum agar containing 1 per cent. of salicin produces a more robust colony than other media. There is always a noticeable tendency for growth to occur in a continuous film instead of in discrete colonies, especially if the agar surface is too moist. The colonies are transparent and faintly opalescent, the contours are smooth or indented, and the edges may be more or less crenated.

Colonies in deep agar shakes are delicate and branching, the centre of the colony is never very compact or well defined.

Meat medium:	Gas; colour varying from bright red to pink; no blackening; odour is rancid but not putrid.
Milk:	Acid and clot; some gas may be formed: the change in the milk is slow; the clot does not as a rule appear before 3 to 6 days.
Coagulated serum:	No liquefaction. The morphology of the organism is very variable.
Alkaline egg broth:	The medium is rendered more opaque; there is no clot.
Gelatine:	Liquefied.
Broth:	The medium becomes turbid: after a period of growth the organisms settle down to the bottom of the tube, leaving a perfectly clear supernatant fluid.

Substances fermented.	Substances not fermented.
Glucose	Glycerine
Laevulose	Saccharose
Galactose	Inulin
Maltose	Mannite
Lactose	Dulcite.
Salicin.	

Animal reactions. Pigeons, guinea-pigs, mice, rabbits, and dogs are all susceptible to infection with *vibrion septique*. Individual strains

vary a little in pathogenicity, but the pathogenic quality is retained unaltered over years of subculture. The lethal dose of a given strain remains fairly constant, provided the same growth conditions are repeated.[1]

The most infective inoculum for experimental animals is a 24 to 48-hour culture in glucose broth with or without a piece of fresh tissue. Ordinary nutrient broth cultures are slightly less infective.

If a lethal dose (0·01 to 0·5 c.c., according to virulence and condition of culture) of a living culture of *vibrion septique* is injected into the muscles of the thigh of a guinea-pig, the animal dies in 12 to 24 hours with oedema and the development of gas in the tissues. Spontaneous recovery in guinea-pigs which have become infected with *vibrion septique* does not occur. Sub-lethal doses produce no symptoms whatsoever, and with *vibrion septique* it may be said that for guinea-pigs an infecting dose is a lethal dose.

On post-mortem examination an extensive blood-stained oedema is observed and a considerable amount of gas is found to have been developed in the areolar tissue around the muscles involved. Pockets of gas are almost invariably seen in the groins and axillae. The muscles affected have a characteristic very intense deep red colour and are softened, but there is no putrid odour.

There may be a collection of fluid in the peritoneal cavity and in the pericardium; this is not as a rule very evident in guinea-pigs which have died in 12 to 24 hours, but is very well-marked in rabbits which have succumbed to the infection after 24 to 48 hours. The suprarenal glands may show a variable amount of redness, but this is not so regular or so marked a feature as in guinea-pigs which have died from infection of *B. welchii*. In mice the local lesion is of the same character as that described for guinea-pigs. The organs do not, however, show any great changes. The adrenal bodies are most frequently affected, and they may show congestion varying in degree from a light pink to a deep red colour.

Microscopic examination of the exudates of animals infected with *vibrion septique* shows very numerous motile rods and usually the characteristic 'navicular' or 'citron' types. Swollen club-ended rods and short chains of intensely staining bulb-shaped individuals may be seen. Spore-bearing rods are, however, not as a rule to be found until some hours after death.

The peritoneal surface of the liver shows long snake-like filaments which are of diagnostic importance. It was this appearance which led to the erroneous idea that the organism was a vibrio. It may be noted, however, that when dealing with mixed cultures of *vibrion septique* and other spore-bearing anaerobes, 'citron' types in the muscles and filaments on the guinea-pig's liver are not always present.

Toxin. A soluble toxin can be demonstrated in 24 to 48 hour broth cultures. (See later, section IV).

[1] The conception of a 'lethal dose' of a living culture is notoriously inexact. It is, however, less misleading with the *vibrion septique* than in most cases. The reason of this is that probably a fairly constant amount of toxin is required to produce the conditions requisite for infection, i. e. for the proliferation of the bacteria in the tissues.

Agglutination. Agglutinins are produced in the blood of rabbits inoculated intravenously with heated and washed cultures of *vibrion septique*. In using monovalent agglutinating sera, it is found that the strains fall into several well-defined serological groups.

Distribution. *Vibrion septique* is said to be present in the intestine of men and animals and in the soil of cultivated land; it is, however, less frequently found in nature than *B. welchii*.

B. chauvoei.

B. chauvoei. This organism is the cause of the disease known as 'Black-leg', 'Quarter ill,' or 'Blackquarter', as 'Charbon symptomatique' by the French and as 'Rauschbrand' in the German literature. There has been a good deal of confusion in regard to this bacillus, although it has been known and studied for a long period. It was probably with impure cultures of this organism that Grassberger and Schattenfroh (1, 2 and 3) carried out their extensive studies which led to conclusions now recognized as being erroneous.

The characters of *B. chauvoei* are very briefly as follows:

It is a very strict anaerobe. Although it has not so far been recorded as occurring in man (see p. 92), it is very pathogenic for mice and guinea-pigs. Rabbits are relatively insusceptible.

Morphology. In broth and meat media, clostridial forms and rods with subterminal spores are to be seen; the spores are of a more elongated oval shape than those of *vibrion septique*. In serum media or in the presence of fresh tissue, navicular forms and club-shaped types are produced. The navicular individuals are, however, longer and more slender than those usually seen in *vibrion septique* cultures. In infected guinea-pigs the navicular and club-shaped elements are also present, but no long filaments are found on the peritoneal surfaces of the liver. This is quite an important feature in the differential diagnosis of *B. chauvoei* and *vibrion septique*.

It is frequently stated that *B. chauvoei* is a Gram-negative organism. In fresh smears from infected tissues and in young cultures the bacillus is definitely Gram-positive. In older cultures Gram-negative individuals may, however, frequently be met with.

Cultural reactions.

Meat Medium:	Gas; pinkish colour which may fade; no putrid odour; no blackening of the medium.
Milk:	Acid; clot in 3 to 6 days; some gas may be evolved.
Coagulated serum:	No liquefaction.
Gelatin:	Liquefied.

Substances fermented.	Substances not fermented.
Glucose	Glycerine
Galactose	Mannite
Laevulose	Dulcite
Maltose	Inulin
Saccharose	Salicin.
Lactose.	

It should be noted that *B. chauvoei* ferments saccharose and does not ferment salicin, whereas the contrary is the case with *vibrion septique*.

Toxin. A specific soluble toxin is produced by *B. chauvoei*.

(iii) *B. oedematiens.* Weinberg and Séguin (1).

This organism, which was discovered by Weinberg and Séguin in 1915 and subsequently identified by Legros (3), Vaucher (1), Dalyell and others, is closely allied to, if not identical with, *B. oedematis maligni II* described by Novy in 1894. It is motile under strictly anaerobic conditions, for instance when examined in a sealed capillary tube; under a cover-slip, however, it is non-motile. In shape *B. oedematiens* is a stout rod as thick as *B. welchii* (0·8 to 1 μ) but usually longer. The rods are frequently curved and the spores which are readily formed in all media are large and oval with slightly flattened ends. They are subterminal or central in position. The morphology of the organism should be studied in young cultures as autolysis sets in very early. There is relatively little variation in the appearances presented by *B. oedematiens* but short chains and filaments are occasionally formed.

Cultural reactions. B. oedematiens requires strict anaerobic conditions to ensure surface growths. Surface colonies are flattened and tend to be confluent forming a translucent film. In agar shakes the growth is delicate, resembling snowflakes, but more solid colonies like conventional bursting grenades may also be seen.

Meat medium:	Gas; pinkish colour which fades quickly; in some samples of meat medium the colour may not change very markedly with growth.
Milk:	After long incubation (10 to 30 days), there is an acid reaction and clot, the acid reaction may be seen in vigorous cultures after 4 or 5 days, but the clot is always delayed.
Coagulated serum;	no change in medium.
Broth:	Early flocculation and sinking to the bottom of the tube where the organisms rest as a semi-opaque cloud (18 to 36 hours).
Gelatine:	Medium is liquefied.
Fermentation:	Production of acid is feeble.

Substances fermented.	Substances not fermented.	
Glucose	Glycerine	Mannite
Laevulose	Galactose	Dulcite
Maltose	Saccharose	Inulin
	Lactose	Salicin.

Animal reactions. The pathogenicity of different strains varies somewhat; guinea-pigs, mice, rats, and rabbits are all susceptible.

The injection of 0·25 to 1 c.c. of a 24 hour broth culture into the thigh muscles of a guinea-pig produces death within 24 to 48 hours. At the post-mortem examination the muscles immediately at the site of inoculation are found to be red, softened but not diffluent. As a rule very little if any gas develops. There is a spreading gelatinous oedema which is usually quite colourless but if the infection has progressed very rapidly the oedema may be tinged with pink. The muscles of the abdominal wall are unaltered in appearance. The bacilli are to be found in numbers only at the site of inoculation. In general the oedema fluid and the peritoneal

surfaces of the liver show only infrequent individuals. Cultures from heart blood are generally positive, except in cases where the animal succumbs to the effect of the toxin present in the inoculum and not to an actual infection with the bacilli.

Toxin. *B. oedematiens* develops an active toxin in broth cultures. (See section IV).

Agglutination. Agglutinins can be produced in rabbits by the intravenous injection of washed bacilli. The sera thus obtained, however, agglutinate only the homologous strain. Agglutination tests must be carefully controlled as many strains show auto-agglutination.

Distribution. *B. oedematiens* has been isolated from samples of earth from cultivated areas by injecting small quantities into the muscles of guinea-pigs protected by means of antitoxic sera against *B. welchii*, *B. tetani*, and *vibrion septique*.

(iv) *B. histolyticus.* Weinberg and Séguin (12).

B. histolyticus holds a position that is intermediate between the acutely pathogenic, invading anaerobes and the secondary purely saprophytic forms.

B. histolyticus is a motile rod very frequently arranged in pairs. It is about 0·5 to 0·8 microns broad and about 3 to 5 microns long. Very young cultures (14 to 16 hours growth) should be examined to obtain information concerning the appearance of this organism. The bacilli degenerate very soon in cultures, and after 24 to 48 hours of growth many Gram-negative individuals may be seen together with fusiform and skittle-shaped involution types. The spores, which are readily formed in all media, are oval and usually sub-terminal; they are considerably wider than the rod in which they arise.

Cultural reactions. Surface colonies are delicate and flat; they have crenated or irregular edges. Colonies in deep agar are arborescent or coral like with fine woolly ends to the branches.

Meat medium:	Digested with production of a white deposit of tyrosin.
Coagulated serum:	Liquefied.
Gelatin:	Liquefied.

Substances fermented.

Glucose
Laevulose
Maltose.

Distribution. This organism is difficult to isolate; it is found in wounds and has been obtained from earth.

The strains of *B. histolyticus* vary very greatly in pathogenicity as tested by injection of young broth cultures (16 to 18 hours) into guinea-pigs. Some strains produce very serious lesions culminating in the death of the animal, while others are only very slightly if at all pathogenic. The bacillus is actively proteolytic and digests living tissue (Weinberg and Séguin (15)). This can be demonstrated by injecting 1 c.c. of a young broth culture of a virulent strain into the thigh of a guinea-pig; in the course of 12 hours the skin and muscles

become digested and a haemorrhagic liquefaction of the soft parts of the limb takes place. This digestion of the tissue may spread over the abdomen and the animal may die in the course of the next 12 to 24 hours or it may recover with a more or less complete necrosis of the limb.

Toxin. A toxin can be demonstrated in broth cultures of 14 to 16 hours' growth, but is destroyed if growth is allowed to proceed beyond 18 hours. It is difficult to filter the toxin successfully as it is largely retained in the candle. The action of the toxin resembles that of *vibrion septique* in that when injected intravenously (in rabbits) there is practically no incubation period, death occurring within five to fifteen minutes. An antitoxin neutralizing this substance has been produced in horses (Weinberg and Séguin).

(v) *B. botulinus.* van Ermengem.

This bacillus was first described by van Ermengem (1) in 1896 in connexion with an epidemic of meat poisoning in Flanders. It has not been found in wounds, and so far as is known is in no way involved in any aspect of the gas gangrene question. A description of its chief characters is appended here for the sake of completeness as it ranks among the highly toxigenic anaerobes.

Although it has not been frequently isolated the recent investigations of E. C. Dickson would indicate that its distribution in nature is wider than was formerly believed.

Morphology. *B. botulinus* is a motile Gram-positive rod, somewhat larger than *vibrion septique* but resembling it in general appearance. The bacilli are usually single but short filaments are not infrequently formed. Spores are not readily produced, but when present are small and oval, usually sub-terminal in position and do not distend the rod to any great extent.

Cultural reactions. *B. botulinus* is difficult to cultivate upon artificial media and requires suitable conditions. The organism will not grow in media the reaction of which is acid. Even the presence of CO_2 inhibits the growth. The best results are obtained by the use of media containing fresh animal tissue or glucose, and growth occurs between 18° C. and 35° C. Good conditions of anaerobiosis are essential. Surface colonies can be obtained upon glucose media. They may attain to 1 mm. in diameter in 24 to 48 hours. They are flat, irregular in shape, and of a greyish colour. If they are examined with a hand lens they are found to be irregularly mottled. Madsen described colonies on glucose gelatine as being circular, transparent, and yellowish in colour; they were composed of coarse granules which are seen to be in continual movement. The gelatine was liquefied round the colony. Upon further incubation the colonies increased in size and sometimes reached several millimetres in diameter. They do not assume an opaque feathery appearance. In deep shakes of glucose agar, semi-opaque biconvex or kidney shaped colonies are produced of about 1 mm. in diameter after 24 to 48 hours' incubation. There is usually a central nucleus and older colonies may send out irregular projections. A considerable amount of gas may be produced.

Meat medium :	Poor growth. This medium is not suitable for the organism.
Milk medium :	Growth is scanty and frequently fails altogether. No change in the medium.
Coagulated serum :	Not liquefied.
Gelatine :	Liquefied.

Substances fermented.	Substances not fermented.
Glycerine	Galactose
Glucose	Saccharose
Maltose	Inulin
Lactose	Mannite
Starch.	Dulcite
	Salicin.

Animal reaction. *B. botulinus* grown in broth, or in broth to which a piece of living tissue has been added, produces a characteristic toxin which causes the death of guinea-pigs within 36 to 48 hours. A tenth of a c.c. of such culture injected subcutaneously into a guinea-pig of 250 grm. causes typical signs of botulismus in 36 hours, there is a complete muscular paralysis, dilatation of the pupils, shallow breathing, intense salivation, and the death of the animal supervenes after a short period. The death is mainly due to the action of the toxin. The organism can, however, be cultivated post mortem from the tissues and the blood of the injected animal (McIntosh).

Animals very susceptible to botulinus toxin are rabbits, guinea-pigs, mice, cats, and monkeys. Madsen using a filtered toxin found that 0·0015 c.c. killed a guinea-pig in 1 to 2 days and 0·0010 c.c. killed in 4 to 5 days. The symptoms he describes are marked muscular relaxation, greenish discharge from the mouth, aphonia, aphagia, constipation, dilatation of pupils, and great loss of weight.

(vi) *B. fallax.* Weinberg and Séguin (5).

This is a motile bacillus which occurs in infected wounds and may be found in cases of gas gangrene. It is occasionally present in blood cultures from the patient. When recently isolated certain strains are pathogenic for guinea-pigs (Weinberg and Séguin (9)), this character is, however, rapidly lost upon cultivation. A strain, which had lost its pathogenic properties for mice, killed however when injected along with a small dose of calcium chloride.

B. fallax is a somewhat slender rod, of about 3–6 microns in length, with rounded ends. It is often slightly curved. Gram-negative elements are frequent and there is altogether rather a feeble capacity to retain the stain. Spores are not readily formed on any media but do occur in small numbers in meat and coagulated serum. They are oval and usually subterminal.

Growth reactions. On surface plates the colonies are round or crenated and slightly granular. Deep colonies are lenticular, irregular or bean shaped.

Meat medium :	Gas ; pinkish colour ; no digestion.
Milk :	Acid clot after some days (3–7).
Coagulated serum :	No liquefaction.
Gelatin :	No liquefaction.

B. fallax ferments glucose, laevulose, and maltose. Some strains also attack galactose. Accounts vary somewhat in regard to the fermentations of *B. fallax*; Henry considering that in addition to the above sugars, galactose, saccharose, starch, inulin, and salicin are also fermented.

Toxin. A soluble toxin is produced.

2. B. Tetani. Nicolaier.

(i) *Bacteriological account.*

Bacillus tetani was described by Nicolaier in 1884 and cultivated by Kitasato in 1889.

It is extremely difficult to isolate in a pure state; many of the cultures obtained from serum institutes and from laboratory collections being found to contain some other anaerobe in addition to *B. tetani*. The contaminating organisms usually belonged to the type of *B. sporogenes*, but oval and round end-sporing bacilli such as *B. cochlearius* and *B. tetanoides*, were also found. It is to the impurity of many of the cultures of *B. tetani* that the great variety in the cultural characters ascribed to this organism is due. (McIntosh, Robertson).

It may be noted that agglutination tests showed that all these laboratory cultures belonged to one serological type (Tulloch (1)).

Morphology. *B. tetani* is a motile Gram-positive rod. In young cultures it is rather a stout bacillus about 4 to 8 microns in length and 0·4 to 0·6 microns in breadth.

The spores are spherical and always strictly terminal. The bacillary rod becomes thinner after the spore is formed but remains attached to it for some time; this condition gives the familiar drumstick appearance. In pure cultures in the ordinary culture media spores are not formed as a rule until the 3rd or 4th day.

Cultural reactions. *B. tetani* is a very strict anaerobe but surface growths can be obtained on serum agar with or without the addition of glucose under good conditions of anaerobiosis.

Surface colonies are flat and delicate, sometimes with finger-like projections. After 48 to 72 hours the centres become raised a little above the surface of the medium and a ground glass appearance may be observed. They are generally small, being not more than 1 mm. in diameter.

In agar shakes (preferably containing glucose) the colonies appear as delicate filamentous outgrowths spreading from a small central nucleus. They may attain a diameter of 2 mm. after 48 hour's growth.

Meat medium:	Pink colour or no change in medium according to the samples of meat; softening of the consistency of the meat. The odour is characteristic but not putrefactive.
Milk:	Poor growth; no change in medium.
Coagulated serum:	Little or no liquefaction.
Gelatine:	Liquefied.

None of the following substances are fermented :

Glycerine	Maltose	Starch
Glucose	Saccharose	Mannite
Laevulose	Lactose	Dulcite
Galactose	Inulin	Salicin.

Animal reactions. *B. tetani* when injected in the form of a broth culture of 3 to 4 days' growth produces death with the characteristic symptoms of tetanic intoxication. This condition is due solely to the absorption of toxin ; the bacilli do not invade the tissues. Washed and heated emulsions of bacilli and spores, that is to say, cultures deprived of their toxin, are not pathogenic.

Toxin. A characteristic toxin is produced by the growth of *B. tetani* in broth.

Agglutination. Washed and heated organisms injected intravenously into rabbits provoke the production of agglutinins in the serum of the animals injected.

By means of the agglutination test the strains of *B. tetani* can be divided into 4 serological groups. This matter along with its bearing on serum prophylaxis is discussed in detail in the following section by Major Tulloch.

(ii) *Recent Experimental Work on B. tetani.*

By W. J. Tulloch, M.D., Bt.-Major R.A.M.C.

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Member of the War Office Committee for the Study of Tetanus.
From the Laboratories of the R.A.M. College and the Lister Institute of Preventive Medicine.

The prevalence of tetanus in the war has been studied statistically by many bacteriologists, Lumière, Chavasse, H. Pribram. In this country the papers published by Bruce (1, 2, 3, 4, 5, and 6), by Leishman and Smallman, and by Cummins and Graeme Gibson, show that, roughly speaking, the disease was comparatively common in the early months of the war, but that its incidence has steadily fallen since the autumn of 1914 when prophylactic inoculation with antitetanic serum was introduced.

The tetanus bacillus can generally be cultivated from the wounds of actual cases of the disease and is by no means uncommon in the wounds of patients showing no clinical evidence of tetanus. Thus, from 100 wounds of such cases examined, true tetanus bacilli were recovered from 19 at one time or another. The bacilli may persist in wounds for long periods, as is shown by the fact that in one case *B. tetani* was obtained 882 days after infliction of the injury.

A brief description of *B. tetani* has been given above and will therefore not be repeated here. Nor will the generally known and well established facts of tetanus be touched upon, attention being devoted to the new experience gained since 1914 and particularly to the researches which have been conducted on behalf of the tetanus committee of the War Office.

A new technique which has been found of great use in dealing with large numbers of cases of tetanus, or supposed tetanus, is described in the Appendix.

One of the most interesting facts established by the study of a considerable number of strains of tetanus bacilli is that there exist at least 4 distinct serological types. Type I is the standard U.S.A. bacillus and is the organism which appears to have been usually employed in laboratories in Europe prior to 1914 for the preparation of antitetanic serum. Types II, III, and IV have been differentiated since.

The relative frequency of occurrence of these types, their value as toxin producers, the similarity or dissimilarity of their toxins and consequently of antitoxins and other evident problems have been studied.

With regard to the first of these points it should be emphasized that, in all probability, most of the anti-tetanus serum manufactured in England and in America in the first years of the war was obtained by the employment of toxin from Type I of the bacillus. If such monovalent serum be more efficient against infection with the corresponding type of bacillus, one would expect that a census of tetanus bacilli found in wounds, from actual cases of the disease among inoculated men, would show a relative preponderance of infections due to Types II, III, and IV.

Of 100 strains of *B. tetani* obtained from cases of tetanus among inoculated men, Type I bacillus was found in 41 instances, Type II in 22, Type III in 33, and Type IV in 4. But of 25 strains of true tetanus bacilli isolated from wounds of men who showed no signs of the disease 76 per cent. were of Type I, 12 per cent. of Type II, 8 per cent. of Type III, and 4 per cent. of Type IV.

This very large discrepancy between the percentage of Type I in the two series is possibly due to the greater efficiency of a monovalent serum against the corresponding type of bacillus. The methods which have been employed in the investigation of this have been both statistical and experimental. On the statistical side an analysis of the series of 100 cases of tetanus has shown that of 91 cases known to have received tetanus antitoxin prophylactically the case mortality in the men infected with Type I bacillus was lowest. This, together with the figures for the other types of bacillus, is shown in the accompanying diagram (Fig. I), which deals only with the 91 cases definitely known to have received serum prophylaxis; in the remaining 9 cases the records are incomplete.

Figure II shows the mortality in cases of tetanus occurring in men inoculated prophylactically with antitoxin. The onset of symptoms occurred within 14 days of the infliction of the wound.

The influence of the antitoxin employed—presumably monovalent Type I—on the percentage occurrence of the different types of *B. tetani* in 'indifferent wounds' and in wounds of actual cases of tetanus, is shown graphically in Figure III.

This statistical analysis suggests that the serological type of the infecting bacillus may be of importance in relation to the pathogenesis of tetanus in inoculated men. To prove this by such methods would require an investigation of a very large number of cases of

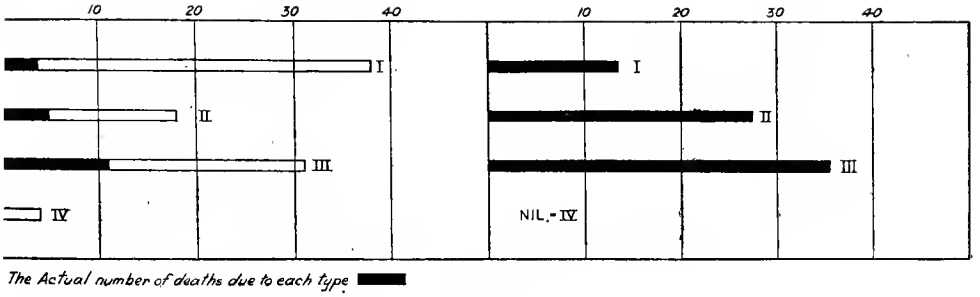


FIG I.

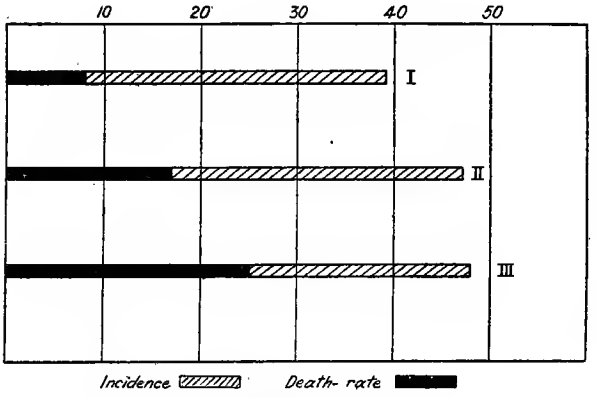


FIG. II.

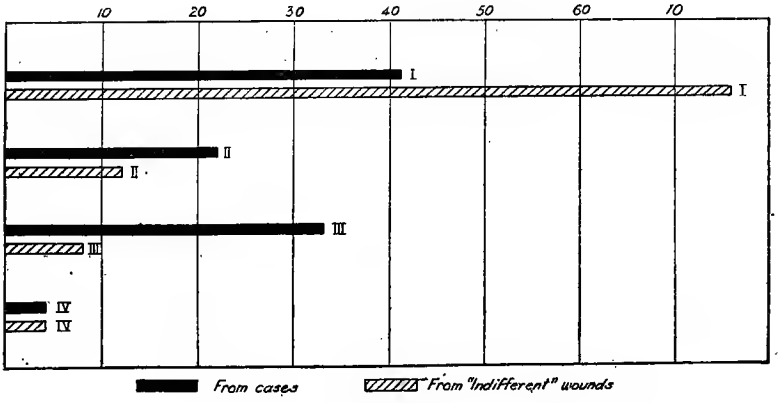


FIG. III.

the disease. The matter was, therefore, submitted to experiment inquiry.

It may be stated at once that the results obtained by exper mental methods, though they are of considerable general interes are inconclusive as to the special point under consideration. Takin first the antitoxins, it has been shown that a mono-typical antitoxi is equally active in neutralizing toxin of any or of all the types c *B. tetani* when the test animals are rats, mice, or guinea-pigs. Bu there is some evidence, not conclusive, that monotypical antitoxi sera may under certain circumstances exhibit specific anti-infectiv properties in relation to the types.

The differences may be bound up in the different types of bacill but it may be said that anti-bacterial sera containing no antitoxi or negligible quantities of it, do not prevent infection in the experi mental conditions under which one is forced to conduct the investiga tion, nor is there so far, any evidence that anti-bacterial sera o a given antitoxin content afford more adequate protection agains infection, than do pure antitoxic sera of the same antitoxin content

This statement is made with reserve as anti-bacterial sera wer not available in sufficient quantity to permit of crucial experiment being carried out.

Infection in Tetanus. Tetanus is rarely produced by the inocula tion of bacilli or spores deprived of their toxin by washing or, in th cases of spores, by washing and heating combined. Susceptibl animals withstand very large numbers of the organisms, 2,000 millio in the case of guinea-pigs and 200 million in the case of mice. The toxin of the tetanus bacillus as ordinarily prepared is feebly aggres sive, differing in this respect from the toxins of the organisms o gas gangrene. When the toxin of *B. welchii*, in sub-lethal doses is inoculated along with washed and heated spores of *B. tetani* the spores germinate and the animal succumbs to tetanus. The toxin of *vibrion septique* acts in a similar manner though not sc regularly. Chemical irritants have been used to initiate tetanus infection in animals. Lactic acid is very uncertain in action; trimethylamine in certain concentrations is able to permit the development of tetanus in guinea-pigs but in mice rarely; saponine will invariably provoke tetanus in guinea-pigs, seldom in mice; calcium chloride, on the contrary, almost invariably induces the development of infection in the latter.

The degree of tissue destruction with lactic acid, trimethylamine and saponine is considerable; with soluble calcium salts it is small.

The quality of tissue ' debility ' at the nidus of infection is, therefore, of great importance, and experiment has shown that the degree and quality of such debilitation is of greater importance than the actual number of organisms injected.

When an insufficient degree of local disturbance is produced in guinea-pigs, typical tetanus, due to infection, occurs, but some of the animals recover and in such circumstance local tetanus has frequently been noted. Cases of local tetanus in experimental animals was also noted, when the degree of tissue debilitation was relatively great, provided, in this instance, that the animals had been

passively immunized with anti-tetanus serum before, or soon after, the injection of the ' spore debilitant ' mixture.

The importance of the symbiotic factor, i.e. the influence of the products of *B. welchii*, &c., in the initiation of tetanus infection is evident. On the one hand it has been shown that the toxin of *B. welchii* may provide the conditions required by the tetanus bacillus in initiating the disease; on the other hand it has been shown that certain mixed cultures from wounds are non-toxic although they contain true tetanus bacilli. From these cultures the tetanus bacillus may be recovered in a sufficient state of purity to permit of its being agglutinated in the presence of type sera. Further, the organisms are not deficient in toxigenic capacity as is shown by injecting into animals the washed spores, derived from such cultures, together with tissue debilitant, when tetanus develops. Moreover, by growing one of the stock cultures of *B. tetani* in the presence of these mixed growths, the toxigenic capacity of the tetanus culture was greatly reduced. The particular combination of organisms which leads to this depression of pathogenicity is not yet fully determined, nor can it be stated whether it is the infectivity or toxigenicity which is depressed, though presumably the latter.

All these facts show that the conditions, under which tetanus may occur naturally, are very complex, and that when the infection has begun factors may come into operation which increase or diminish the violence of the disease.

An inquiry into surgical procedures in relation to degree and persistence of Anaerobe Infection of Wounds.

This investigation, which was carried out by Miss Cayley, indicated that

(*a*) None of the antiseptics investigated could be specially recommended as valuable for the elimination of anaerobic infection.

(*b*) Anaerobic bacilli, even those of pathogenic significance, may persist in wounds until the completion of the process of repair.

(*c*) The degree of anaerobic infection of wounds that have been excised is, on the whole, less than in those that have not been so treated. While excision therefore does not eliminate infection it does alter the condition of the wound in such a fashion that the harmful capacity of any anaerobes present is much reduced.

(*d*) In examining the influence of brilliant green and other aniline dyes on growth of anaerobes *in vitro*, it was found that their activity was very much reduced when the cultures were made by Tarozzi's method. This reduction of activity is more marked than that caused by the presence of serum. This observation has a direct bearing on the application of aniline dyes as antiseptics in surgical therapeutics.

Technique.

In making the investigations summarized above, the following technique was used.

Swabs taken from wounds—whenever possible these were taken from the deeper parts—were emulsified in about 3 c.c. of saline.

Of this, 1 c.c. was inoculated into ordinary meat water medium and incubated anaerobically—Culture A. This gave an index of the non-sporing anaerobic bacilli present.

The remainder—2 c.c.—was heated to 80° C for 10–15 minutes or to 60° C for 30–40 minutes.

Of this 1 cc. was inoculated into a tube of meat water medium and incubated anaerobically—Culture B. This gave a growth of the sporing anaerobes present.

The 1 cc. which remained was inoculated into a tube of ' selective ' medium designed to give overwhelming growth of endsporing bacilli —Culture C.

The selective medium was prepared thus :

Take 1 lb. of chopped meat, add 1 litre of tap water, and boil for 30 minutes. Cool to 45° C., make slightly alkaline to litmus and add trypsin as for the preparation of Douglas' broth ; then incubate in an open vessel for 4–5 days at 37° C. allowing the material to undergo natural putrefaction. Pass the putrescent material so obtained through paper pulp to clear, and make neutral to phenolphthalein at room temperature. The material is then sterilized by filtration through a Berkfeld and a Doulton candle in series. The medium is stored in sterile flasks under a layer of paraffin to which sodium formate has been added to the extent of 1 per cent. of the total volume of medium. The flasks should be provided with a hooded delivery tube, so that the material may be distributed as required. It keeps fairly well—about three weeks—but should never be sterilized by heat.

Before use the sterility of the medium should be tested by inoculating quantities of from 5 c.c. to 0·1 c.c. into tubes of meat water medium and incubating anaerobically for at least 7 days. When the medium is to be employed for cultures that may ultimately be used for animal inoculation, it must be shown that the medium itself is non-toxic. Before use, fresh sterile rabbit kidney is added to the medium, $\frac{1}{16}$th part of a kidney being sufficient for 5 c.c. The tubes to which kidney has been added should be used within 3 days.

Prepared thus, the medium inhibits the growth of *B. sporogenes* but allows of the growth of *B. tetani*, atoxic round endsporing bacilli, and certain oval endsporing bacilli.

Latterly, in place of using meat water tubes for culture A and B, the following medium has been employed :

The flesh of one rabbit is chopped, 1 litre of water containing 5 grams sodium carbonate is added, and the mixture is allowed to decompose for 16–24 hours at 37° C. This mixture is then examined and the reaction again adjusted to be slightly alkaline to litmus, and 2 per cent. of trypsin added. The flask is now returned to the incubator for a further period of 16–24 hours. The material is then filtered through paper, made slightly acid, and boiled to coagulate proteins, filtered again and neutralized. In neutralizing, the mean of two titrations is taken at room temperature (the specimens for the tests having been previously boiled and rapidly cooled) (1) with phenolphthalein and (2) for α-naphtholphthalein, and the requisite amount of sodium hydrate is added to the bulk of the medium. Boil again and finally filter. The medium may then be tubed and

autoclaved or autoclaved and stored in bulk. Before use, $\frac{1}{16}$th part of fresh sterile rabbit kidney is added to tubes of 5 c.c. Tubes which have been stored should be boiled for 30 minutes and rapidly cooled before the kidney is added. The medium should be used as soon as possible after the addition of the kidney. This medium gives very heavy growths of *B. tetani* and appears to have some, though not marked, selective properties.

Tubes A, B, and C were examined at two days' intervals and when C showed a growth consisting mainly of endsporing bacilli the culture was centrifuged, washed, and tested by the agglutination technique. Not infrequently culture C failed to show growth, although round endsporing bacilli might ultimately develop in tube A or tube B. In such cases A or B or both A and B were heated to 60° C. for 45 minutes, and subcultures made from them into the selective medium. In this way a number of cultures of *B. tetani* could be obtained, although they failed when directly inoculated into the selective medium.

The technique of the agglutination test and that of the preparation of agglutinating sera are described fully in papers dealing with the serological differentiation of *B. tetani*.

3. Anaerobes the Action of which may be Ancillary to the Condition of Gas Gangrene.

The less directly pathogenic anaerobes which proliferate in wounds comprise a very large number of diverse types. It is proposed to describe only the more frequent and important species. It is not claimed that even this list is complete; the forms included are, however, those which have appeared with sufficient regularity to be considered of some definite importance in wound infection. It is admittedly difficult to estimate the rôle that these ancillary organisms play in gas gangrene and in the very much more frequent local infections caused by anaerobic bacteria.

Blood culture from the patient occasionally reveals the presence of some of these types, notably *B. sporogenes*, but the fact that organisms can be cultivated from the patient's blood is not necessarily significant when wounds of large area are present. In pure culture they are practically all to be classed as non-pathogenic for laboratory animals. With few exceptions soluble toxins have not been found in cultures of any of these organisms but in no case so far have they been exhaustively examined for this property.

In mixed cultures they are for the most part, though not invariably, favourable to each other's growth, and it is presumed that they act with an equally successful mutual assistance in wounds.

It is well-known that it is not until two or three days after the infliction of the wound that the anaerobic flora of this mixed, less pathogenic type, arises. Some of the characters of these infections, such as putrefactive odour, digestion, and gas formation can be explained from the known reactions of organisms isolated from these cases. These sub-acute conditions may show one or several of the pathogenic anaerobes already dealt with and this mixed type

of infection is always a state that may proceed to acute gas gangrene or to acute tetanus.

In wounds in which these sub-acute local infections have subsided and also in late septic wounds without obvious local symptoms of anaerobic action, many of these bacterial types linger on, especially when sequestra or adherent sloughs are present, multiplying sufficiently to be very readily cultivated from quite small samples of wound exudate. It is not precisely known what, if any, importance these organisms have in weakening the tissue resistance and in delaying the healing process, but it is probable that their local action is unfavourable to the patient.

Pathogenic anaerobes may proliferate as apparently harmless saprophytes, but their presence, even as spores in sequestra and in the scars of healed wounds, are a source of great potential danger especially when disturbed by injury or surgical interference.

(i) *B. sporogenes.* Metchnikoff.

This organism was first described by Metchnikoff, 1908, who distinguished two varieties or races; 'A' derived from the faeces of healthy persons, and 'B' derived from persons suffering from chronic colitis. He distinguished them chiefly by their morphological characters, A being more slender than B. Both races have filamentous woolly colonies and agree with each other in cultural characteristics.

B. sporogenes as found in wounds appears to belong to Metchnikoff's race A.

It is present in a very large proportion of wounds, and appears in acute cases of gas gangrene as well as in conditions in which the wound is progressing in a satisfactory manner. It has been cultivated from the blood of the patient during life, sometimes alone, but more often in association with other bacteria.

B. sporogenes is widely distributed in nature; it is frequently found in earth and in practically all materials exposed to dust. It is a common inhabitant of the alimentary canal of man and animals and can be cultivated from a great variety of sources including milk. The organism is extremely tenacious of life as is shown by its capacity to survive 8 days in a 5 per cent. phenol solution (Schütze), or an exposure in a capillary tube to 100° C. for 45 minutes.

The name *B. sporogenes* is used in a wide sense and the strains designated by this title are capable of further sub-division upon minute characteristics.

Morphology. B. sporogenes is an actively motile rod of about 3 to 7 μ in length; it closely resembles *vibrion septique* in appearance and is more slender than *B. welchii*. Spores are very readily formed in all media, are oval in shape and central or subterminal in position. The range of bacillary forms is more restricted than in the organisms already described; long filaments being the only variation. The bacilli are Gram-positive, but in old cultures Gram-negative individuals are found.

Cultural reactions. B. sporogenes is an anaerobic organism, but it

does not exact a perfect condition of anaerobiosis ; it grows readily on agar plates in an exhausted cylinder.

Colonies. Surface colonies of *B. sporogenes* are characterized by the production of woolly tangled filaments which grow out all round the more solid centre and down into the medium. Young colonies are very transparent and irregular in shape ; later, they become opaque and woolly with a definite raised centre. Old colonies may be yellowish in colour. They have a tendency to grow large and are solid in type. Isolated colonies on thinly sown plates may attain a diameter of several millimetres. Deep colonies are woolly.

Meat medium :	Vigorous growth ; gas ; alkaline reaction ; digestion of the medium and blackening ; the extent of the change of colour varies with different samples of the meat medium. Putrid odour.
Milk :	Digestion occurs without the production of a firm clot, leaving a somewhat turbid supernatant fluid : the reaction becomes alkaline.
Coagulated serum :	Liquefied and becomes darker in colour.
Alkaline egg broth :	There is a flocculent precipitate or even a soft clot which sinks to the bottom of the tube leaving a clear supernatant fluid ; the clot is digested to greater or less extent.
Gelatine :	Liquefied.
Sugars :	Glucose, laevulose, and maltose are alone fermented.

Microbic association. One feature of the behaviour of *B. sporogenes* calls for particular notice, namely, its extraordinary capacity for persisting in the presence of other organisms. Only bitter experience can convince workers, unaccustomed to the clinging and pervasive character of this bacillus, of the extreme difficulty of dislodging it from the cultures of any other organism with which it has become associated. *B. sporogenes* will linger unsuspected for weeks or years in a culture of *B. welchii* or of *vibrion septique*, only to appear when some particularly favourable set of circumstances (such as an alkaline protein medium) permits it to obtain the upper hand in the association. Moreover *B. sporogenes* will fall into a commensal adjustment with some other organism such as *B. tetani*, *B. tertius*, *B. cochlearius* or *vibrion septique*, lending its putrefactive and proteolytic characters to these types and thus leading to great confusion. These mixed cultures will keep a consistent appearance in regard to type of colony and cultural reactions over long periods, and it is only by ringing the changes over a wide range of media and conditions that the observer has reason to suspect the composite character of the strain which he believed to be pure.

This difficulty of freeing anaerobes from one another is not confined to *B. sporogenes*, but there is no doubt that it is the most frequent and most persistent intruder. The tendency to grow in mixed colonies, the power of cryptic proliferation within the characters of another species without being detectable, and the capacity of mutual adjustment possessed by anaerobes and by *B. sporogenes* in particular are at the root of nearly all the more serious confusions and discrepancies in the literature of this subject.

Animal reactions. In general *B. sporogenes* cultures are not lethal for laboratory animals in doses up to 4 c.c. Some strains are, however, capable of producing a putrid perforating gangrene of the limb injected and a small number of strains are described as being definitely lethal in doses of about 1 c.c. and upwards.

The presence of *B. sporogenes* in combination with *B. welchii* quite definitely enhances the pathogenicity of the latter and produces a mixed putrefying type of gangrene closely resembling the putrefying gangrene of wounds which was of frequent incidence in the earliest days of the war. *B. sporogenes* added to a sub-lethal dose of *B. welchii* produces a virulent and rapid type of gangrene in experimental animals. In this condition an extensive oedematous swelling of the limb is observed a few hours after inoculation. The skin takes on a shiny blistered appearance, and a curious green colour. The hair is generally shed over the blistered parts. Death may supervene at this stage. If the animal survive for twenty-four hours the skin over the oedema becomes sodden and perforates, with the production of a foetid sloughing gangrene of the limb. The animal may die after the gangrene has set in or it may gradually recover even in cases where a considerable amount of tissue has become necrosed. The post-mortem examination of a guinea-pig, dead from a mixed infection of *B. welchii* and *B. sporogenes* shows the following features. The skin of the abdomen is soft and greenish, and the hair is easily detached if it has not already come out before death; there is an extensive fluid oedema, blood-stained and oily in appearance, which extends as a rule over the whole abdomen up to the axillary region. Haemorrhagic patches are sometimes to be observed in the subcutaneous tissues remote from the lesion. The site of the inoculation is oedematous; the tissues are friable and are dark in colour. There is some gas formation; the odour is foetid and distinct from that produced by *B. welchii* alone. The parietal peritoneum is purple and the blood-vessels are injected. Lungs, liver, and spleen are unchanged in appearance.

The injection of *B. sporogenes* with *vibrion septique* gives less certain results, but the general effect is the same.

B. sporogenes therefore cannot be neglected in the gangrene syndrome though it may be difficult or impossible to gauge its effect or importance in any given case.

Non-specific toxic products of B. sporogenes. The growth of several of the non-pathogenic anaerobic bacilli, in particular that of *B. sporogenes*, is accompanied by the production of toxic substances. These are for the most part of a non-specific nature and appear to be formed as the result of the breaking down of the food material. Without doubt, similar substances are produced by the pathogenic anaerobes in addition to the formation of specific toxins. The exact relationship of these two kinds of products is not very clear, but the non-specific products lead to much of the confusion concerning the presence of the specific bodies. In certain instances their presence together with the great lability of the specific toxins masks more or less completely the presence of the latter.

The demonstration of the non-specific toxic substances is best

made by growing the bacilli in a meat medium, as suggested by Besson. This is prepared by placing minced meat in a flask with enough water to cover it. Several cubic centimetres of soda (1 per cent.) are added and the medium is then sterilized at 115° C. for 15 minutes. If this is inoculated before it is completely cold the anaerobes will grow quite well if the cotton-wool plug be replaced by a rubber stopper.

The maximum toxicity seems to be developed about the sixth day, after which it gets rather less. The fluid is decanted off the meat, the juice expressed, and the two fluids mixed and filtered.

Toxicity. The toxicity of such a fluid varies considerably, but as a rule from 1 c.c. upwards injected into the peritoneum of a young guinea-pig will produce almost immediate signs of intoxication which are not infrequently followed by a fatal issue. If death does not occur in the course of a few minutes the animal usually recovers completely. After the injection of a lethal dose the animal immediately becomes restless, jumps about, jerking its head upwards in a peculiar manner, then becomes convulsed and falls over on its side. The symptoms observed are therefore quite distinct from those which follow the intraperitoneal injection of fatal doses of the true anaerobic toxins.

Nature of the poison. Dale and Barger stated that the poisonous substance present passed over when the media was distilled. An analysis of the distillate convinced them that they had to deal with a volatile poison probably of the nature of an ammonium base.

A series of experiments by McIntosh and Fildes suggested that some sulphide—probably ammonium sulphide—played an important part; the presence of sulphides in the cultures can readily be demonstrated, and the injection of weak solutions (0·5 c.c. of a $\frac{1}{2}$ per cent. sol.) of ammonium sulphide produces symptoms of immediate intoxication and even death.

The influence of the growth products of *B. sporogenes* upon the action of the toxin of *B. welchii* has been studied in mice (W. E. Bullock and Cramer (1 and 2)). When a mouse has been injected with a sub-lethal dose of sterile toxin of *B. welchii* together with sterile filtrate from a culture of *B. sporogenes*, in an amount of either substance insufficient of itself to produce symptoms, the animal dies.

Specific toxin. A true soluble toxin has not as yet been demonstrated for *B. sporogenes*.

Agglutination. Agglutinins can be produced in the blood of rabbits. So far as is at present known strains of *B. sporogenes* can be divided into at least two serological groups by means of this test.

(ii) *B. parasporogenes.* McIntosh.

This organism was originally isolated and described as Type XII by J. McIntosh (1917). It agrees with *B. sporogenes* in all its characters with the exception of two. In the opinion of the committee these differences, which are quite constant, are considered to be of sufficient importance to make it advisable to place the organism in a separate species and the name parasporogenes is adopted.

B. parasporogenes differs from *B. sporogenes* in the nature of its

colony ; in agar shakes it is lenticular or slightly irregular in shape but not woolly.

The second feature which distinguishes *B. parasporogenes* is the production of specific agglutinins. In morphology and in cultural characters and in all other respects the two organisms resemble each other closely.

(iii) *B. tertius.* Henry, 1917 [1] (Rodella's Bacillus III) (von Hibler's Bacillus IX).

B. tertius is a saccharolytic oval endsporing anaerobe. It is found both in early and in late wounds and may be present in severe cases of gas gangrene, or in ordinary wound infections.

Morphology. B. tertius is sluggishly motile. The rods are slender and about 3 to 5 microns in length, they are Gram-positive when young (12–18 hours), but soon tend to become Gram-negative especially in fluid media. Spores are readily formed, and as stated, are oval and strictly terminal. The morphology of the organism is best studied in cultures 24 to 36 hours old.

Cultural reactions. B. tertius requires only a moderate degree of anaerobiosis for growth.

Surface colonies are small (1 mm.) and are rounded or crenated ; when young (24 hours) they are delicate and almost transparent ; examined under a hand lens by transmitted light they have a characteristic greenish-blue iridescent appearance not at all unlike a small opal. On certain media they become more opaque and slightly granular when incubated for two or three days.

Colonies in agar shakes are small, lenticular or irregular in shape and do not show branching filaments.

Meat medium :	Pink colour ; gas ; no blackening ; no digestion.
Milk :	Acid clot in 3 to 6 days ; some gas.
Coagulated serum :	Not liquefied.
Gelatine :	Not liquefied.

Substances fermented.	Substances not fermented.
Glucose	Glycerine
Laevulose	Inulin
Galactose	Dulcite.
Maltose	
Saccharose	
Lactose	
Mannite	
Salicin.	

(iv) *B. cochlearius.* Douglas, Fleming, and Colebrook (3).

This organism was first described as type III C. by McIntosh, 1917.

It is an endsporing bacillus very frequently found in wounds. Like *B. tertius* it may be present both in acute early infections and in late septic cases. It is frequently associated with *B. tetani.*

[1] The name tertius was given by Henry because it was the third most common micro-organism in his series.

Morphology. *B. cochlearius* is an actively motile slender rod; the length is variable, and there is a tendency to give up the stain in Gram's method. The spores are strictly terminal and are oval when fully developed, giving the organism a spoon-shaped appearance, hence the name *cochlearius*. There are stages, however, during their growth in which the young spores may be almost spherical. In perfectly pure cultures on ordinary media spores are not readily formed. The morphological features can be well observed in an alkaline digest broth containing fresh tissue, as this medium is particularly favourable to the formation of spores. The examination should be made after 24–36 hours' incubation. An impure culture containing a slight admixture of *B. sporogenes* gives a vigorous growth of *B. cochlearius* with active sporulation—an instance of stable microbic association between two organisms. Such a culture resembles *B. putrificus* (Bienstock) and has frequently been designated by this name. It is proteolytic, digests meat and inspissated serum and gives off a putrid odour. It is often extremely difficult to dissociate *B. cochlearius* from *B. sporogenes* in such a mixed culture.

Cultural reactions. In pure culture *B. cochlearius* is one of the less vigorous anaerobes. Surface colonies can be obtained upon serum agar plates or serum agar slopes under good conditions of anaerobiosis. They are delicate, glass-clear droplets, sometimes with faintly crenated edges. When colonies show minute opaque spots or opaque centres or have rootlets penetrating into the medium it is a sign of admixture with some other anaerobe, probably *B. sporogenes*. Colonies in agar shakes are lenticular in shape.

Meat medium :	Very little change in the colour. There is no digestion and very little gas. There is a characteristic odour which is not putrid.
Milk :	Scanty growth ; no change in medium.
Coagulated serum :	Not liquefied.
Gelatine :	Not liquefied.

None of the following substances are fermented :

Glycerine	Maltose	Mannite
Glucose	Saccharose	Dulcite
Laevulose	Lactose	Salicin.
Galactose	Inulin	

Agglutinins can be produced in rabbits by the inoculation of washed bacilli.

The inoculation of living cultures of *B. cochlearius* intramuscularly into guinea-pigs in doses up to 3 c.c. does not produce pathogenic results.

B. cochlearius is distinguished from *B. tertius* by its failure to ferment any of the sugars, by its motility, characteristic odour, and the production of agglutinins in the blood of rabbits.

(v) *B. tetanomorphus.*

This is the organism described by McIntosh and Fildes as ' Type IX, *B. pseudotetanus* '. It closely resembles *B. tetani* in its morphological

appearance; but differs in cultural characters, its power to ferment maltose and glucose, the absence of liquefaction in gelatine and in its inability to produce the specific toxin. It is frequently found in wounds, especially in late infections, and is often present along with *B. tetani*.

Morphology. *B. tetanomorphus* is highly motile; the rods are Gram-positive. The spores are spherical, terminal in position, and are formed in all media in the course of 24–36 hours.

Growth reactions. *B. tetanomorphus* grows best in an alkaline medium. When a sample of wound exudate containing *B. tetanomorphus* is incubated in meat medium the organism makes its appearance in numbers about the 3rd to the 5th day, that is to say considerably later than *B. sporogenes*. The conditions produced in the medium by the growth of *B. sporogenes* are favourable to *B. tetanomorphus*.

Surface colonies can be obtained upon serum agar slopes and plates. They are delicate, flat, and have a slightly crenated edge. There is a tendency to grow in a continuous surface film. Colonies in deep agar are small in size and irregular in shape but are not woolly or branched.

Meat medium:	Pink colour; gas; no digestion or blackening. (This is the best medium for the growth of *B. tetanomorphus*.)
Milk:	No change; scanty growth.
Gelatine:	No liquefaction.

Substances fermented.	Substances not fermented.	
Glucose	Laevulose	Lactose
Maltose.	Galactose	Inulin
	Saccharose	Glycerine
	Salicin	Dulcite.
	Mannite	

Agglutinins for *B. tetanomorphus* can be demonstrated in the serum of rabbits inoculated intravenously with the bacillus. *B. tetanomorphus* is not pathogenic for guinea-pigs.

(vi) *B. aerofetidus.* Weinberg and Séguin (8).

Morphology. This is a small slender bacillus about 3–5 μ in length; it has a tendency to be Gram-negative. Motility is slight.

Growth reactions. Spores are not readily formed; they are subterminal. Surface colonies on serum are round and transparent and may attain to 1 or 2 mm. in diameter after 24 hours' incubation.

Deep colonies are small and irregular.

Meat:	Putrid odour; change of colour, first reddening and then blackening.
Milk medium:	Clot and gas in 24–48 hours, later, a certain amount of digestion.
Coagulated serum:	Putrid odour; liquefaction.
Gelatine:	Liquefied.
Alkaline egg broth:	Soft clot which may be partially digested.

This organism is not pathogenic in pure culture for guinea-pigs.

Fermentation. There is some divergence of opinion as to the sugars fermented by this organism. Glucose, maltose, and lactose were, according to McIntosh, the only sugars fermented, whereas Henry (2) found that laevulose and salicin were also affected.

(vii) *B. bifermentans.* Tissier and Martelly.

This bacillus was first described by Tissier and Martelly in 1902 under the name of *B. bifermentans sporogenes.* It received the name of *bifermentans* owing to its being the first anaerobe in which a capacity to split both proteins and sugars was definitely recognized.

B. bifermentans is occasionally found in acute cases of gas gangrene, as well as in late septic cases.

Morphology. In the non-sporing state it is a stout, non-motile rod closely resembling *B. welchii.* Individual elements may be very short. Chains are frequently formed. Spores are readily formed in all media ; they are central or subterminal.

Cultural reactions. Surface colonies are round or crenated. Deep colonies are lenticular or irregular but without filamentous outgrowths.

Meat medium :	Blackened and digested ; putrid odour ; gas.
Milk medium :	Casein is precipitated and later digested.
Coagulated serum :	Liquefied.
Gelatine :	Liquefied.
Alkaline egg broth :	Soft clot which is later digested to a variable extent.

Fermentation. Glucose, laevulose, and maltose are alone fermented. *B. bifermentans* is non-pathogenic in pure culture for guinea-pigs. Agglutinins can be produced in rabbits inoculated with the organism.

(viii) *B. putrificus.* Bienstock (2).

This organism was first described by Bienstock in 1899 under the name of *B. putrificus.* He and many subsequent writers consider that it is strongly proteolytic. Experience during the war has shown that many of the cultures of *B. putrificus* so-called are really mixtures of *B. cochlearius* or *B. tertius* with *B. sporogenes.*

The following is a brief synopsis based upon the incomplete descriptions of Bienstock, Salus, Tissier and Martelly, Metchnikoff, Würcker, Weinberg and Séguin, and others.

B. putrificus is a slender Gram-positive rod, the spores are oval and strictly terminal giving the drumstick appearance described by Bienstock.

The cultures are strongly proteolytic ; gelatine and serum are liquefied and milk digested with or without the formation of a clot.

The organism is described as being non-pathogenic (Bienstock, Tissier and Martelly).

(ix) *B. sphenoides.* Douglas, Fleming, and Colebrook (3).

This is a small motile bacillus, Gram-positive when young but rapidly becoming Gram-negative. In the non-sporing state it is

fusiform in shape and often arranged in pairs placed end to end not unlike a Hoffmann's pseudodiphtheria bacillus.

The spore when first formed is subterminal, but as it increases in size its position becomes terminal; it is perfectly round and is of large size, being considerably broader than the body of the bacillus. As the spore reaches its maximum development the body of the bacillus assumes a wedge shape the point being situated at the end opposite to the spore. It was the appearance of the bacillus at this stage which suggested the name sphenoides. As the body of the bacillus degenerates in older cultures, the organism becomes shaped like a drumstick, but the end opposite the spore always remains pointed.

Cultural reactions. Surface colonies on agar attain to a size of about 1 mm. in diameter. They are round and usually have smooth edges; occasionally, however, slight irregularities are seen.

Meat medium:	A little gas is formed; no change is produced in the colour.
Milk medium:	Acid is formed and occasionally a soft clot.
Coagulated serum:	No change.
Gelatine:	Not liquefied; good growth.
Broth:	The growth is profuse, an even turbidity being produced.

The fermentation reactions of this organism appear to be somewhat variable. Glucose, maltose, galactose, lactose, and salicin are, however, fermented by the three strains isolated. Two of the strains ferment mannite, saccharose, dextrine, and starch in addition.

Incidence. It was isolated in pure culture from three cases and was observed in two others in a total of sixty-one (Douglas, Fleming, and Colebrook (3)). The majority of the patients had had gas gangrene.

(x) *B. butyricus.*

B. butyricus is a non-pathogenic bacillus rarely met with in wounds. In 1861 Pasteur (1) described, under the name of 'Vibrion butyrique', an anaerobe which was capable of producing butyric fermentation. This organism of Pasteur is probably identical with the *Clostridium butyricum* of Prazmowski, the *Bacillus amylobacter* of Gruber and with the mobile 'Buttersäurebacillus' of Grassberger and Schattenfroh (1 and 2).

The literature dealing with the organism is full of confusion and it is almost impossible at the present date to decide exactly with what organisms the above authors worked. The characters of what the committee consider to be *B. butyricus* are detailed below from the study of a single strain isolated by Fleming from a wound.

Morphology. It is a small Gram-positive motile bacillus. The spores are small and oval in shape, usually central though occasionally subterminal in position. The most frequent appearance is that of an elongated clostridium.

Cultural reactions. Surface colonies on serum-agar are small, flat, irregular in shape, greyish in colour and semi-transparent. Shake colonies are small irregular lenticular masses.

Meat medium :	No digestion ; some gas.
Milk medium :	A firm acid clot appears in 24 hours.
Coagulated serum :	No change in the medium.
Gelatine :	Not liquefied.

Fermentations. Glucose, maltose, lactose, saccharose, and starch are fermented.

(xi) *B. multifermentans tenalbus.* Stoddard (3), 1919.

This is a non-pathogenic organism recovered from a case of gas gangrene. It is an infrequent organism but is of interest as it shows a morphological resemblance to *vibrion septique.*

Morphology. It is a Gram-positive motile bacillus. The spores are central or subterminal. When grown upon egg or serum media, navicular types, filaments, and swollen club-shaped individuals are formed.

Cultural reactions. Surface colonies, cultivated under anaerobic conditions, may attain a large size (3 to 5 mm.) ; they are round, with slightly irregular edges ; after incubation for several days they become white and opaque and rise up considerably above the surface of the agar. In glucose-agar shakes the colonies are white and opaque, irregular or lenticular in shape with projecting outgrowths.

Meat medium :	Gas ; no obvious digestion ; no blackening ; the odour is not putrid.
Milk medium :	Acid and clot.
Coagulated serum :	No change.
Gelatine :	Not liquefied.

Substances fermented.		Substances not fermented.
Glycerine	Glucose	Mannite
Maltose	Saccharose	Dulcite.
Lactose	Inulin	
Raffinose	Salicin.	

4. Classification of the Anaerobic Bacilli found in Wounds.

The characters available as a basis for the classification of the anaerobic bacilli can be grouped under the following headings :

Morphology.
Cultural characteristics.
Serological reactions.
Production of characteristic toxin.

Each of these categories deals with a different aspect of the organism, and owing to the difficulties inherent in the study of bacteria no one of these aspects taken by itself furnishes the data for a satisfactory classification. Since, therefore, a combination of these several different series of characters has to be relied upon, the order and relative value of the different criteria must be considered.

(i) *Morphology.*

The first subdivision is based upon morphology and the next upon cultural reactions. The production of characteristic toxins is a quality

which is valuable in classification where available, and serological reactions form a final series of distinctions which may or may not be of specific value.

The anaerobes may be divided into three groups upon their morphology :

(*a*) Organisms with oval spores which are central or sub-terminal in position.

(*b*) Organisms with oval spores which are strictly terminal in position.

(*c*) Organisms with spherical spores which are strictly terminal in position.

(ii) *Cultural Characteristics.*

Further divisions can be made upon cultural reactions such as the capacity for decomposing protein, carbohydrates and alcohols.

All organisms have a certain capacity for attacking proteins and splitting sugars, but these characters have to be sufficiently marked to be readily appreciable under the test-tube conditions of artificial cultivation and have to be somewhat arbitrarily defined for the purposes of classification.

In the case under consideration proteolytic characters are judged by the capacity of liquefying coagulated serum and of gelatine. In working with the anaerobes the gelatine test has been used in the special manner first employed by Rüneberg and differs from that generally employed in bacteriology. Owing to the unsatisfactory amount of growth of the anaerobes obtained at 25° C., the inoculated gelatine stab is incubated at 37° C. for forty-eight hours under anaerobic conditions, thereafter it is cooled by being placed in a beaker of cold water and the reading taken. In the case of the saccharolytic organisms the fermentation is recognized by a definite capacity for producing acid or acid and gas in sugar-containing media.

Subsections based upon these characters can be arranged as follows:

A. Organisms showing proteolytic and saccharolytic properties.

B. Organisms showing proteolytic properties only.

C. Organisms showing saccharolytic but no proteolytic properties.

D. Organisms showing no obvious proteolytic or saccharolytic properties.

Subsection A would be subdivided into :

A 1. Organisms predominatingly proteolytic with, however, a restricted but definite capacity of fermenting certain carbohydrates.

A 2. Organisms predominatingly saccharolytic possessing, however, in addition, slight proteolytic properties as shown by the liquefaction of gelatine.

The criterion in accordance with which an anaerobe is here classified as a predominatingly proteolytic organism is the capacity to produce liquefaction of coagulated serum.

The accompanying table shows the arrangement of the organisms classified in these terms.

CLASSIFICATION.

	Both proteolytic and saccharolytic properties.		*Slight proteolytic but no saccharolytic properties.*	*Saccharolytic but no proteolytic properties.*	*Neither saccharolytic nor proteolytic properties.*
Morphology	Proteolytic properties predominating. Coagulated serum and gelatine are liquefied.	Saccharolytic properties predominating. Serum not liquefied gelatine liquefied.	Serum not liquefied. Gelatine liquefied.	Neither serum nor gelatine liquefied.	Neither serum nor gelatine liquefied.
Central or subterminal spore.	*B. sporogenes.* *B. parasporogenes.* *B. histolyticus.* *B. aerofetidus.* *B. bifermentans.*	*B. welchii.* *Vibrion septique.* *B. chauvoei* *B. oedematiens.* *B. botulinus.*		*B. fallax.* *B. butyricus.* *B. multifermentans tenalbus.*	
Oval terminal spore.				*B. tertius.*	*B. cochlearius.*
Spherical terminal spore.			*B. tetani.*	*B. tetanomorphus.* *B. sphenoides.*	

TABLE OF THE CHARACTERS OF THE ANAEROBES ISOLATED FROM WOUNDS.

Name.	*Motility.*	*Spores.*	*Surface Colony.*	*Colony in Agar shake.*	*Cultural Reactions in*				*Animal Reactions.*	*Remarks.*
					Milk.	*Meat.*	*Coagulated Serum.*	*Gelatine.*		
B. sporogenes.	+	Oval: central or subterminal. Spores are readily formed in all media; they are very resistant to heat.	Woolly colony with tangled filaments at the periphery.	Opaque woolly colony.	Precipitation of casein which is later digested.	Gas: blackening: digestion with very putrid odour	Liquefaction.	Liquefaction.	Not pathogenic for laboratory animals: large doses may produce local necrosis: a few strains are said to be pathogenic for guinea-pigs.	Non-specific toxic products are produced in culture media.
B. paraspo-rogenes.	+	As for *B. sporogenes.*	Round and opaque. Occasionally the margin shows woolly filaments later.	Opaque colonies, lenticular or irregular.	As for *B. sporogenes.*				Not pathogenic for laboratory animals.	
y-	+	Oval; usually subterminal. Spores are readily formed in all media.	Flat, delicate colonies with crenated edges.	Arborescent coral-like colonies, with fine woolly ends to the branches.	Digested.	Digested. Tyrosin-like crystals produced.	Liquefied.	Liquefied.	Strains vary in pathogenicity. Pathogenic strains produce haemorrhagic liquefaction of the soft parts of the limb injected; death occurs in 24 to 48 hours.	Specific toxin produced.
-	Slight.	Oval and subterminal. Spores are not readily formed in any medium.	Round, transparent, with feathery processes.	Small irregular masses.	Clot and gas in 24 to 48 hours, later, a certain amount of digestion.	Putrid odour: medium becomes red then blackens.	Liquefied.	Liquefied.	Not pathogenic.	

B. bifermentans.	—	Spores are usually central but may also be subterminal. They are readily formed in all media.	Round or crenated.	Lenticular or irregular but without filamentous outgrowths.	Precipitation of casein, which is later digested.	Gas: blackening: digestion with putrid odour.	Liquefied.	Liquefied.	Not pathogenic.	Morphology is characteristic. Stout rod often growing in chains. The spores do not distend the rod.
ii.	—	Large oval with slightly flattened ends; central or subterminal. Spores formed only in sugar free media rich in protein, such as coagulated serum, alkaline egg fluid and casein broth.	Round with smooth edges.	Opaque and lenticular.	Stormy fermentation: very rapid clotting with evolution of gas: clotting torn with gas: acid reaction.	Gas: pink colour: sharp butyric acid odour: no blackening.	Not liquefied: spores formed: filaments and involution types occur.	Liquefied.	Pathogenic for guinea-pigs, pigeons and mice: less so for rabbits. Spores not formed in the animal: no long filaments on surface of liver. Many strains are of low pathogenicity.	Produces a specific soluble toxin.
e.	+	Spores are formed readily in all media: they are central or subterminal.	Delicate and faintly opalescent: round sometimes with indented or crenated edges.	Semi-transparent with fern-like branchings.	Acid and clot: some gas may be formed: slow reaction, 3 to 6 days.	Gas: pink colour which fades later: no blackening.	Not liquefied: variations in morphology—citrons, navicular types, club-shaped forms, &c. may be developed.	Liquefied.	Pathogenic for guinea-pigs, pigeons, rabbits and mice: long threads are formed on the peritoneal surface of the liver. Citrons and navicular types, &c. may be seen in the tissues post mortem.	Produces a specific soluble toxin.
B. chauvoei.	+	Spores are formed readily. They are central or subterminal.	Delicate round or with spreading indented edges.	Colony small, composed of minute club-like filaments.	Acid: clot in 3 to 6 days: some gas may be evolved.	Gas: pink colour which fades later: no blackening.	Not liquefied: navicular forms, &c. may be developed.	Liquefied.	Pathogenic for mice and guinea-pigs: rabbits are relatively insusceptible; long filaments are not formed on the peritoneal surface of the liver. Navicular types may be seen in the tissues post mortem.	Distinguished from *vibrion septique* by sugar reactions, specific agglutinins and specific soluble toxin.

TABLE OF THE CHARACTERS OF THE ANAEROBES ISOLATED FROM WOUNDS—*Continued.*

Name.	*Motility.*	*Spores.*	*Surface Colony.*	*Colony in Agar shake.*	*Cultural Reactions in*				*Animal Reactions.*	*Remarks.*
					Milk.	*Meat.*	*Coagulated Serum.*	*Gelatine.*		
B. oedematiens.	Only motile under strictly anaerobic conditions.	Spores formed in all media: oval with slightly flattened ends: central or subterminal.	Flat and inclined to be confluent, growing out in finger-like processes.	Transparent snow-flake colonies, sometimes with more opaque centres.	Acid after 4 to 6 days. Slight clotting after some weeks.	Gas: no blackening: pink colour at first, then bleached.	Not liquefied.	Liquefied.	Pathogenic for mice, guinea-pigs and pigeons: produces gelatinous oedema. Does not usually form spores in animal body, no long filaments on liver.	Produces specific soluble toxin.
B. botulinus.	+	Spores are formed but not readily: they are oval and subterminal.	Flat, round or irregular.	Semi-opaque, bi-convex or kidney-shaped: older colonies may send out irregular projections.	No change in medium, growth is scanty and may fail.	Poor growth: medium is unsuitable.	Not liquefied.	Liquefied.	Pathogenic for laboratory animals.	Produces specific soluble toxin. This organism is difficult to cultivate upon artificial media. Growth occurs between 18° C. and 35° C.
	+	Spores are not formed readily, but do occur in small numbers in meat and coagulated serum: they are oval and subterminal.	Round or crenated and slightly granular, occasionally contained in a bubble.	Lenticular or irregular.	Acid: clot after 3 to 7 days.	Gas: pinkish colour: no blackening.	Not liquefied.	Not liquefied.	When recently isolated some strains are pathogenic for guinea-pigs, the pathogenicity disappears upon cultivation.	This organism is sometimes recovered from the blood of the patient in cases of gas gangrene. Produces a soluble toxin.

B. butyricus.	+	Oval spores usually central but may also be subterminal.	Flat and irregular in shape.	Irregular or lenticular.	Firm acid clot in 24 hours.	Gas: no blackening.	Not liquefied.	Not liquefied.	Not pathogenic.	
B. multifermentans tenalbus.	+	Spores are central or subterminal.	Round with slightly irregular edges.	Irregular or lenticular: colonies are opaque.	Acid and clot.	Gas: no blackening.	Not liquefied.	Not liquefied.	Not pathogenic.	
B. tertius.	– or only very faintly motile.	Spores formed readily: they are oval and strictly terminal.	Round and opalescent with slightly crenated edges. May be granular when older.	Irregular or lenticular: opaque.	Acid and later clot.	Gas: no blackening.	Not liquefied.	Not liquefied.	Not pathogenic.	This organism is actively saccharolytic which distinguishes it from *B. cochlearius.*
B. cochlearius.	+	Spores are formed but not readily: they are oval and strictly terminal.	Round, smooth or slightly crenated glass-clear colonies.	Lenticular.	No change.	Little gas: no blackening.	Not liquefied.	Not liquefied.	Not pathogenic.	Growth is not prolific in pure culture.
B. tetani.	+	Spherical and strictly terminal.	Flat and delicate with projections growing out at the edges.	Branching and flocculent.	No change.	Gas: no blackening.	Not liquefied.	Liquefied.	Pathogenic.	Produces a specific soluble toxin.
B. tetanomorphus.	+	Spherical and terminal.	Flat with slightly crenated edges.	Irregular but do not grow out into branches.	No change.	Gas: no blackening.	Not liquefied.	Not liquefied.	Not pathogenic.	
B. sphenoides.	+	Spherical and terminal when fully developed.	Round, usually with smooth edges.		Acid and sometimes a soft clot.	Little gas: no change in colour.	Not liquefied.	Not liquefied.	Not pathogenic.	There is a characteristic wedge-shaped appearance in the sporing state.

(iii) *Agglutination Reactions.*

At a very early period in the history of anaerobic bacteria, it was found that the injection of the bacilli into animals led to the production of agglutinins. Thus Leclainche and Morel prepared a serum which agglutinated *vibrion septique* in dilutions of 1 in 500 and upwards, while Leclainche and Vallée (1) prepared a serum which agglutinated *B. chauvoei* in dilutions up to 1 in 3,000. These writers claimed that the sera produced were rigorously specific. About the same time the production of specific agglutinins against *B. tetani* was demonstrated by Courmont. Markoff (1911) showed that agglutination was of considerable value for the differentiation of certain anaerobes, in particular of ' Rauschbrand ', malignant oedema and ' Geburtsrauschbrand ' and almost identical results were obtained by K. F. Meyer.

The more recent researches dealing with the identification of the various anaerobic bacteria of wounds, has again directed attention to the question of agglutinins. Amongst those who found that the agglutination reactions were sufficiently specific to be of diagnostic value may be mentioned, Gaehtgens, Landau, McIntosh, H. C. Plaut, Pfeiffer and Bessau, Robertson and Weinberg and Séguin. Fürth, on the other hand, was much less successful. The consensus of opinion is, therefore, that the agglutination reactions of the majority of anaerobes with the exception of *B. welchii* and *B. tertius* are of great value. According to the above writers the test may be used not only for the differentiation of species but also for the differentiation of sub-species.

Agglutinating sera are best prepared by intravenous injection into rabbits, surface cultures or the centrifugalized deposit of broth cultures suspended in saline should be used for the injection. Rabbits are relatively insusceptible to these emulsions and a whole serum agar slope may be injected. Usually three or four injections made at three or four days interval are necessary to produce a high titre serum.

It is of course essential that the cultures used to prepare the agglutinating sera should be absolutely pure. Failure to observe this will lead to endless confusion and worry.

The agglutination test is best carried out in small test-tubes or in Pasteur pipettes according to Wright's method, immersed in a water bath at 56° C. The bacterial suspensions should be obtained from young cultures, well washed in saline and diluted to give slightly opalescent emulsions. As a rule living bacilli are more readily agglutinated than those killed by heat. Readings are taken after two hours.

The established facts in regard to the more common anaerobes are as follows :

1. *B. welchii.*

Werner (1905) prepared agglutinating sera against four ' Gasbrand ' strains and an original Fraenkel strain. Agglutination was obtained with the homologous strains in dilutions varying from 1 in 200 to 1 in 1,000. No agglutination was obtained on cross agglutination tests. Simonds (1) stated that he prepared a rabbit serum which agglutinated the homologous strain to 1 in 80. One other strain

was agglutinated to 1 in 40, ten strains to 1 in 20 and ten strains not at all. Weinberg (1918) obtained serum from two horses which agglutinated the homologous strains in 1 in 500 and 1 in 2,000 respectively, but he does not state the action of these sera on other examples of the organism.

Bull (1917) mentions the agglutination and lysis of *B. welchii* by normal rabbit and guinea-pig sera and also agglutination in immune sera but has given no exact details of his results. Gaehtgens (1917) found he could prepare agglutinins to all his anaerobes with the exception of *B. welchii*. Pfeiffer and Bessau claimed to have produced a serum against *B. welchii* which agglutinated the homologous strain in 1 in 40. This serum, however, had no effect on fourteen other strains of *B. welchii*. McIntosh (1917) found it impossible to produce definite agglutinating sera against any of his *B. welchii* strains.

It may be assumed from the above evidence that the agglutination test is of no value in the diagnosis of *B. welchii*.

2. *Vibrion septique.*

As previously mentioned, this was one of the first anaerobes for which specific agglutinins were demonstrated. Weinberg and Séguin (18) in titrating a monovalent agglutinating serum against nine strains of *vibrion septique* isolated from human cases found that four of these (including the homologous culture) were agglutinated in a dilution of 1 in 1,000, eight in 1 in 500 and one in 1 in 10 only. The same serum agglutinated a type strain of *vibrion septique* from the Pasteur Institute collection 1 in 500 and a stock strain of so-called *B. chauvoei* from the same source in 1 in 10 only.

More recent research upon the agglutination reactions of *vibrion septique* strains has shown that there are at least three serological types, which while producing different agglutinins are found to be a single group as regards their toxin antitoxin reactions. (Robertson.)

3. *B. oedematiens.*

Weinberg (18) has prepared rabbit sera which agglutinate the homologous organism 1 in 100, but have no action on other strains of the organism. The organism is a difficult one to work with, because most strains tend to agglutinate spontaneously.

B. tertius. Attempts to obtain an agglutinating serum from rabbits for this organism have failed.

4. *B. fallax.*

The following table of agglutination reactions is given by Weinberg (18).

Strains.	*Serum* 1 *rabbit.*	*Serum* 3 *rabbit.*	*Serum* 4 *human.*
1	1/500	1/100.	1/50
2	1/50	0	1/50
3	0	1/500	0
4	0	0	1/100
5	0	0	0

None of the strains of *B. fallax* dealt with were agglutinated by sera prepared against *B. welchii*, *V. septique*, *B. oedematiens*, *B. sporogenes* and *B. aerofetidus*, nor did the *B. fallax* sera agglutinate any of the above mentioned anaerobes.

5. *B. sporogenes.*

Metchnikoff in his original article on *B. sporogenes* described two types but did not mention agglutinins. McIntosh (1917) by means of agglutination tests found at least three different types.

The following table illustrates a series of experiments undertaken with four strains of *B. sporogenes* which were indistinguishable morphologically or culturally (Henry).

Sera.	*Strains.*			
	1	2	3	4
Normal rabbit (5)	—	—	—	—
Normal sheep (1)	50	50	—	—
Normal horse (3)	—	—	50	50
McIntosh (rabbit)	—	—	500	500
Weinberg (rabbit)	—	—	200	200
Rabbit (against 3)	—	—	4,000	2,000
Rabbit (against 4)	—	—	4,000	4,000
Rabbit (against 1)	4,000	4,000	—	—
Rabbit (against 1)	8,000	8,000	—	—
Sheep (against 1)	64,000	32,000	—	—
Goat (against 1)	250,000	64,000	—	—
F 8.	2,000	2,000	50	—
T 8.	500	500	—	50
M 8.	1,000	500	—	50
O 8.	1,000	1,000	—	—

F 8, T 8, M 8 and O 8 were German gas gangrene sera prepared apparently by the inoculation into horses of mixed whole cultures of anaerobes.

A glance at the table shows conclusively that the four strains of *B. sporogenes*, which were taken at random for the experiment, may be divided into two serological groups, each of which is distinctly demarcated from the other in the possession of the capacity to produce a specific agglutinin.

An additional experiment with eleven other strains of *B. sporogenes* showed that of these only two came into the 1–2 group and only one into the 3–4 group. In the case of the remaining eight strains there was no agglutination. One of the latter, a strain isolated from a case of haemothorax was agglutinated by the patient's own serum and by his serous pleuritic exudate, both collected during convalescence, in a titre of 1 in 2,000.

The agglutination reaction would appear, therefore, to divide the *B. sporogenes* group of organisms into a number of sub-groups.

6. *B. tetani.*

Tulloch (1) has shown that the strains of *B. tetani* he investigated are divisible on their serological reactions into three groups.

Summary.

The application of agglutination as a diagnostic procedure in the case of the anaerobes results in a differentiation which may be said to reveal evidence of tribal rather than of national characteristics.

Where an anaerobe can be shown to be pathogenic, its capacity to produce a toxin which presents well-defined characters provides an unassailable basis for classification. Hence, in dealing with a group or collection of strains, the individual members of which have in common the property of producing one and the same toxin,

any further division into sub-groups that results from the application of an agglutination test leads to an unwarranted multiplication of separate bacteriological types. In this respect the agglutination test as applied to the anaerobes becomes ultraspecific.

(iv) *Toxin-antitoxin Reactions.*

B. welchii, vibrion septique and *B. oedematiens* produce specific toxins, each of which has its own particular characters, and each of which, when inoculated into animals, gives rise to specific antitoxins.

A well-washed emulsion of *B. welchii* may be introduced in large doses into experimental animals without producing infection. The combination, however, of a few washed bacilli with a sub-lethal dose of toxin results in the production of a rapidly fatal gas gangrene. The toxin, therefore, is endowed with powerful aggressive properties. This aggressive action of *B. welchii* toxin can be successfully neutralized by *B. welchii* antitoxin, but not by other antitoxins. One is thus enabled to say quite definitely that if the toxin produced by an unknown anaerobe is completely deprived of its lethal effect in animals when it is mixed with, for example, *B. welchii* antitoxin, then the organism in question is *B. welchii*. A simpler method of demonstrating the same fact consists in mixing a liquid culture of the organism with varying doses of antitoxin before inoculation into animals. If the infection be inhibited with *B. welchii* antitoxin then the organism is *B. welchii*. Or again, if it can be shown that the inoculation of an animal with a *B. welchii* antitoxin renders it passively immune to infection with a particular organism, then one can be certain that the organism is *B. welchii*.

The same is true for *vibrion septique* and for *B. oedematiens*; the toxin of each gives rise to a specific antitoxin which is capable not only of neutralizing the corresponding toxin, but also of inhibiting the infection of experimental animals by whole cultures of the organisms in question.

This method of arriving at an exact diagnosis of the nature of an unknown pathogenic anaerobe is applicable both to pure cultures and to mixed cultures.

The question of toxin-antitoxin reactions is dealt with in detail in the serological section of this Report.

5. Biochemistry.

It is to Spallanzani in 1776 that we owe one of the first, if not the very first, real inquiries into the behaviour of living organisms in the absence of oxygen.

In a set of experiments which are a model of experimental technique, he exposed to a vacuum animalculae in small tubes, sealed at one end. Similar control tubes were left in the air. The experiments were continued for 24 days, at the end of which time he observed that the animalculae in the anaerobic tubes were dead, while those exposed to air survived.

Other organisms were not so susceptible to privation of oxygen, for he recounts that certain animalculae lived 35 days *in vacuo*,

and others died in 14, 11, and 8 days. Some were only able to withstand oxygen want for 48 hours.

He was able to observe that motility and the power of reproduction was not totally inhibited by a vacuum, but he believed that deprivation of air eventually stopped both these processes.

He also showed, in the case of some organisms with which he worked that a partial vacuum was favourable to their development. He thus anticipated by more than a hundred years the work of subsequent investigators on oxygen minima. This astonishing investigation appears to have been entirely overlooked by all the later workers in this field.

(i) *The Mechanism of Anaerobiosis.*

Pasteur in 1861 recognized that certain organisms were able to exist and multiply in the absence of free oxygen. This belief in the possibility of protoplasm continuing to function under strictly anaerobic conditions has always encountered a certain amount of scepticism, but it may be said that the arguments advanced against the original Pasteur conception do not carry great weight with later workers.

The matter of oxygen tolerance is one of a large number of gradations in the animal kingdom, and is therefore analogous to many other processes connected with life and growth. At the upper end of the scale one encounters animals which exist normally in an atmosphere containing twenty volumes per cent. of oxygen, but which can tolerate for long periods pressures of oxygen up to three atmospheres, or concentrations of oxygen as low as seven per cent. Above and below these pressures the animal rapidly succumbs. Other animals, such as *Spirostomum*, one of the ciliates, are killed by the partial pressure of the oxygen in the atmosphere, but exist normally at one-third of this pressure.

With bacteria all gradations of oxygen tolerance are found. Some species cannot live without a certain partial pressure of oxygen, while others, the facultative anaerobes, are capable of living in or without the presence of this element. The obligate aerobes are distinguished by the fact that continued absence of oxygen leads eventually to impairment of function and cessation of growth.

From these forms, the transition to one which does not require free oxygen for its existence is a natural one. In this case oxygen acts as a poison. For this toxic action we have an analogy, higher up in the scale, where a certain concentration of oxygen is necessary for life but a greater one is fatal.

Much careful work has been done in establishing the fact that anaerobic bacteria live in the complete absence of oxygen. To this may be added the consideration that no amount of reserve oxygen present in the cell would suffice to carry on such an intense metabolism as is often found during the growth of these organisms.

Whether the optimum growth of anaerobic bacteria takes place in complete absence of oxygen is still a matter for discussion. The elaborate series of investigations which were undertaken by Fermi and Bassu would lead one to believe that a certain small partial

pressure is necessary for intensive growth. These authors made many experiments to determine the conditions under which the strictest anaerobiosis was obtainable. It seems probable, judging from their experiments, that the ordinary anaerobic technique never gives a medium completely free from oxygen. Another important point is disclosed by their research. While it is possible to obtain growth in the complete absence of oxygen, this is not so abundant as when a very small amount of the gas is present. Whether the small amount has a stimulating action, such as is seen with other poisons, is not known.

In the case of some anaerobes, the oxygen tension at which growth takes place is very low. Chudiakow has shown that in the case of *Bactridium butyricum* the limits are between 0 and 15 mm. At 10 mm. of oxygen there was already a retarded growth. By way of comparison he gives an interesting account of the behaviour of a strict aerobe, *B. subtilis*. This bacterium can grow at 10 mm. of oxygen, but ceases to do so at 5 mm.

The question of 'symbiosis' in the growth of anaerobes is undoubtedly an important one in soil infected wounds. A certain amount of work has been done, which is not for the present purpose very satisfactory. The subject is a difficult one but ought to yield most useful results if further investigated.

One of the earlier ideas regarding 'symbiosis' was that aerobes abstracted oxygen from the medium, and so made conditions suitable for anaerobic growth (Pasteur (2 and 4)).

Kedrowsky claimed that aerobes elaborate certain substances favouring the growth of anaerobes, and that the absorption of oxygen by the former is not an important process. Scholtz and von Oettingen criticized Kedrowsky's results and showed that no special substance was necessary. The idea of Pasteur was modified in such a way that von Oettingen came to the conclusion that the aerobe acted as does the presence of reducing substances in a medium. The anaerobe has an incomplete capacity for oxidation. When it is in company with a micro-organism which is eager for oxygen, the latter acts synergetically and promotes growth. The action of the aerobe is therefore a secondary one. Von Oettingen's technique was ingenious but not beyond criticism.

The question of the smallest quantity of oxygen necessary for growth was taken up by Beijerinck, who concluded that a very small amount of free oxygen always took part in anaerobic growth. Using his experiments as a basis, he proposed to replace the terms anaerobic and aerobic by micro-aerophilic and aerophilic.

The experiments of Kürsteiner, who used *B. phosphoreum* (Cohn) Molisch, as an indicator for free oxygen seem to point to the conclusion that even the smallest quantity of oxygen is not at all essential for the vigorous growth of strict anaerobes, and in this respect his results confirm the earlier experiments of Chudiakow. An excellent critical review of this much debated question is given by Omelianski, who does not believe that free oxygen is necessary. Omelianski also points out the important influence of the composition of the medium on the degree of anaerobiosis necessary for the growth of individual organisms.

A full discussion on the mechanism of anaerobiosis will be found in the papers of Fermi and Bassu, von Oettingen, Beijerinck, Omelianski, and Kürsteiner (cf. Bibliog.).

The chemical reactions which take place during the growth of anaerobes are manifold, and may roughly be divided into attacks on carbohydrates and the decomposition of proteins. In the fermentation of carbohydrates certain compounds are formed of great theoretical and technical importance, acetone, alcohols, especially butyl alcohol and acids of the fatty series. The acid encountered in dealing with anaerobes found in wounds is chiefly butyric acid. This is formed according to the reaction

$$C_6H_{12}O_6 = C_3H_7COOH + 2H_2 + 2CO_2.$$

This reaction may, however, have many by-products, and quite appreciable quantities of other fatty acids are formed at the same time. Non-volatile fatty acids such as lactic acid are formed in quantity.

All the micro-organisms so far examined conform in general to the above scheme. The attack on albuminous compounds is a much more complicated affair, and individual differences between the anaerobes are more manifest.

Roughly speaking they all exert a certain amount of tryptic action and break down proteins to amino acids and ammonia. The so-called saccharolytic organisms *B. welchii* and *vibrion septique* have not this power to any great extent, and therefore one finds the particles in a culture such as cooked meat retaining their original appearance for long periods of time. An analysis shows, however, that a certain amount of digestion has taken place, and it is obvious that such large quantities of gas as are found in cooked meat cultures must have their origin from some type of protein cleavage.

Apart from purely hydrolytic action whereby albumoses, peptones, and amino acids are formed from the original protein molecule, other processes take place, and it is noteworthy that both reductions and oxidations may be occurring at the same time. It is due to these simultaneous chemical reactions that the whole chemical mechanism of anaerobic metabolism is rendered so difficult to disentangle.

The chemical processes may be divided into three groups (Ellinger).

1. Reduction. Replacement of the amino group by hydrogen. In this way phenyl, oxy-phenyl, and indol propionic acids are formed from phenyl alanine, tyrosine, and tryptophane. The general type of equation is the following:

$$R.NH_2COOH + H_2 = R.H.COOH + NH_3.$$

2. The elimination of carbon dioxide with the formation of amines:

$$R.NH_2COOH = CO_2 + R.H.NH_2.$$

3. The oxidative degradation of amino acids whereby the chain is shortened. At the same time the amino group is removed. For example, phenyl acetic acid may be formed from phenyl alanine

$$R.CH_2.CHNH_2.COOH + O_2 = R.CH_2COOH + CO_2 + NH_3.$$

Apparently in these three groups of chemical reactions the reductions take place with compounds having an aromatic nucleus. The oxidations are principally confined to the aliphatic amino acids.

Very little indeed is known regarding the properties of individual bacteria for producing definite types of reactions. Experiments to determine this must be done by bacteria of undoubted purity of strain on definite chemical compounds. In the case of anaerobes, few of the experiments which have been hitherto performed will bear scrutiny.

The chemical mechanism by which the necessary energy is produced for anaerobic metabolism is not an economical one. E. Buchner showed that for *B. subtilis* it was necessary to use up 4·7 times as much material to produce a given amount of heat when grown under low tension as when the organisms grew in the presence of air.

In ordinary aerobic metabolism, carbon dioxide is usually the result of the combustion of the carbon atom, the most efficient heat producing reaction into which carbon enters. In anaerobic metabolism, associated with the formation of this gas, the carbon dioxide is derived from the breaking down of a carbon chain with the simple elimination of carbon dioxide from a carboxyl group, or the formation of an acid of lower molecular weight, or the production of an amine or an alcohol. These are reactions associated with comparatively little heat set free.

(ii) *The Biochemistry of certain micro-organisms found in Wounds.*

A certain amount of chemical work has been done during the war on both the obligate and facultative anaerobes found in wounds. Greater attention has been paid to the former class. The main results are to be found in the papers of Wolf and his co-workers (cf. Bibliog.).

The directions in which biochemical investigations have taken place are two-fold, viz. those on the nature of the toxic products which are formed and those concerned with the growth metabolism of the organisms.

Previous to the war, most of the chemical work on anaerobes dealt with those concerned in putrefaction. A certain amount of information was gathered in the studies of the souring of milk and the ripening of cheese.

Recently the bacterial chemistry of soils, of silos, and of manures, has contributed not a little to our knowledge of the metabolism of anaerobic bacteria, and it is obvious that this branch of agricultural bacteriology is closely allied to the biochemistry of wound infection, for it was from the heavily manured fields of France and Flanders that most of the pathogenic anaerobes were derived. The later chemical investigators used pure strains of anaerobes for their researches and they were, therefore, at a definite advantage over earlier workers who were unable to do so. The work of Nencki, Tissier, and others on putrefaction gives a composite picture of the chemical changes taking place in an albuminoid medium under the influence of putrefactive and other anaerobes, while later work assigns to each organism a definite function in the process. Hence it is that the foundations of our knowledge of the metabolism of the

individual bacteria are based on the methods which have been elaborated for the isolation of pure cultures.

Surgeons have come to recognize three important processes taking place in gas gangrene infections which are capable of being studied biochemically.

The first of these is gas formation, for with it there are obvious physical signs of the type of infection. The production of gas in the tissues is moreover important, for it leads to disturbances of nutrition, whereby the progress of the infection is materially accelerated. It may be said at once that all anaerobes are gas formers under suitable conditions; furthermore that all anaerobes are capable of producing gas in a protein medium like cooked meat. The capacity for gas formation varies with the individual organism, and is, in some cases, largely dependent on the presence of available carbohydrates.

The second sign of gas gangrene infection is that of proteolysis, as denoted by the breaking down of the tissue and the odour, which is sometimes one of the first diagnostic signs encountered in wounds.

All anaerobes are proteolytic, in that they are capable of transforming a certain amount of protein into ammonia and substances liberating nitrogen when treated with nitrous acid.

The differences between the individual species are, however, very great. On the one hand, there are organisms of the type of *B. welchii* and *vibrion septique* in which the proteolysis can only be followed by refined chemical methods; on the other, *B. sporogenes* and *B. histolyticus* produce as profound a disintegration of protein as that produced by trypsin. Indeed the reaction is a more far reaching one, for with the breaking up of highly complex nitrogenous groups to amino acids, deamination and other types of reactions occur whereby large quantities of ammonia are formed. *B. oedematiens* is rather more proteolytic than *vibrion septique* especially if incubated in protein media at 37° C. for a long time. The proteolytic action has not the practical significance of that of *B. sporogenes* or *B. histolyticus*, which, under favourable conditions, will transform a damaged muscle into a stinking pulp within 48 hours. *B. oedematiens* has the power, as its name implies, of producing an alteration in the tissues leading to the exudation of a clear gelatinous oedema. The chemical investigations which have been made on the growth of this micro-organism have, however, not thrown any light on this peculiar change.

It is worthy of note that the truly proteolytic organisms are not those which produce potent toxins. The attack on proteins, or the synthetic process which gives rise to toxin formation must be of a very special kind, and is not associated with any great protein destruction. Experiments which have been made indicate that toxin formation takes place under certain limited conditions especially with regard to the reaction of the medium. The ' fragility ' of some of the toxins produced is directly due to the action of the hydrogen ions upon them, when the hydrogen ion concentration of the nutrient medium is higher than that which exists at absolute neutrality.

The third sign is the general toxaemia seen in gas gangrene. Three classes of toxic effect due to the products of bacterial meta-

bolism, have been distinguished. The first is the true toxic effect, and this varies widely both in degree and in nature.

Some of the anaerobes, e.g. *B. sporogenes*, produce no toxin. With *vibrion septique* a toxin can be prepared of which 0·25 c.c. injected intravenously will kill a large rabbit in 5 minutes. *B. oedematiens* produces a toxin of which 0·003 c.c. subcutaneously will kill a mouse (of 15 gr.) within 24 hours. The details of the toxicity of these products are given in the serological section.

The second type of product which has been thought to cause toxic symptoms is the ammonia. This is certainly formed in large quantity by organisms such as *B. sporogenes* and *B. histolyticus* but it seems improbable that a toxic alkalosis develops as a result.

The third toxic effect has been assumed to be due to the acids formed during the metabolism of these organisms. This view has occupied a prominent place in the discussion of the toxaemia of gas gangrene. Large quantities of acids, especially volatile acids, are formed in cultures of some of the organisms. Whether in large infections of muscle tissue the amount produced is sufficient by itself seriously to deplete the reserve alkali is a matter which has not as yet been fully demonstrated. A. E. Wright and Fleming have shown that a fall in alkaline reserve takes place and have looked upon this as the result of acid production in the wound itself. There are, however, so many factors concerned in the diminution of reserve alkali that it seems unjustifiable for the present to assume that acid formation in wounds occupies a principal place in the production of the toxaemia.

Acid production in culture. All anaerobes, so far examined, produce volatile and fixed acids in the course of metabolism. It is highly probable that this is a property of all micro-organisms, in which behaviour they correspond to other forms of cell life.

As might be anticipated where large amounts of organic acids are formed from amino acid complexes, ammonia is simultaneously produced in the process of deamination and reduction. The ammonia is utilized to preserve the neutrality of the medium, and this is one of the reasons why cultures remain viable in media like cooked meat or alkaline egg, when in fluids containing carbohydrate they are destroyed owing to the formation of acids without the corresponding quantity of ammonia. This is quite apart from any question of spore formation.

In some instances, e.g. the cultures of *B. sporogenes*, the equivalent formation of ammonia and organic acids results in the practical stabilizing of the reaction of the medium. Absolute cessation of growth does not take place for weeks after inoculation. With other organisms, where the acids prevail over the ammonia, and the reaction of the medium progresses in an acid direction, growth stops by reason of the ultimate acidity of the medium. The point at which growth ceases is of theoretical and of possible practical importance.

Taking a medium of a given constitution and given initial reaction, it is always found that the final reaction at which growth stops is constant for each organism. There are slight differences in the final reaction if the initial reaction is varied, but the variation in

the final reaction, especially when available carbohydrate is present, is so small as only to be discovered when the hydrogen electrode is used.

The other point which is of practical importance is that growth of these organisms is limited by an acidity which is approximately that of one thirty thousandth normal solution of hydrochloric or sulphuric acid.

Attention has been paid almost exclusively to the volatile acid formed during growth ; this is so because of the technical difficulties of performing serial determinations of the fixed organic acids. Earlier work has shown that a number of these latter acids are present, but the amount has not been determined. Recent work (Wolf and Telfer), revealed that in cultures of *B. sporogenes* and *B. welchii* on milk and on 2 per cent. glucose peptone broth, 30 per cent. to 50 per cent. of the total acid production consists of non-volatile acids.

Of the fixed acids, lactic acid undoubtedly forms a considerable part, but other acids, such as succinic, phenylacetic, and p.oxy-phenyl propionic are undoubtedly present.

All the earlier qualitative work was done with impure cultures and therefore cannot be accepted without reserve.

The Biochemical Characteristics and their Bearing on the Determination of Species. The work which has been done on the biochemistry of these organisms leads one to inquire whether the available data are sufficient to determine the identity of bacteria which have not been resolved by the usual cultural and morphological methods.

Harris, in a paper which is now in the press, has made for this purpose a careful study of two organisms about the individuality of which some uncertainty exists.

A comparison was made between a typical strain of *B. sporogenes* and the ' Reading ' bacillus, isolated from a wound by Donaldson. The cultural and morphological features of the two organisms were identical.

The results of the work show that in every chemical characteristic the two organisms were the same within the limits of present experimental error. In only one respect did they differ. The oxygen tolerance of the ' Reading ' bacillus was low. It is a very strict anaerobe. The strain of *B. sporogenes*, however, tolerated large partial pressures of oxygen in liquid media, although both behaved as strict anaerobes in surface growths. Whether the difference is a real criterion of individuality it is impossible to say, but it is certain that a large field of investigation is open to anyone who cares to examine the characteristics of closely related strains by the chemical method.

The Chemical Characteristics of Anaerobic Bacteria. So far, a detailed study has been made of those bacteria which are present in wounds and are considered clinically important. These are *B. sporogenes, B. welchii, vibrion septique, B. histolyticus*, and *B. oedematiens.* To these has been added a study of various strains of *B. proteus* as accessory organisms in wounds.

Of the above mentioned, *B. sporogenes* and *B. histolyticus* are

strongly proteolytic. *B. welchii* and *vibrion septique* are feebly proteolytic, but attack certain carbohydrates with great vigour.

B. oedematiens appears to stand by itself. When the quantity of medium is large and the observations are prolonged, it produces considerable quantities of gas in glucose and lactose media, and also in cooked meat when available free carbohydrate is not present. At the same time its proteolytic activities are not inconsiderable in media free from sugars.

Bacillus sporogenes (Metchnikoff). This organism is characterized by the violent attack which it makes on proteins and on carbohydrates. Its proteolytic action, especially in a medium which is rich in protein is very great. In a cooked meat medium containing 0·87 per cent. of nitrogen, 71·5 per cent. will be changed to ammonia and amino acid nitrogen in the course of 286 hours.

It forms large quantities of gas from solutions containing available carbohydrates, over 1,000 c.c. per litre being formed from milk. The amount of gas formed from a 2 per cent. glucose peptone may be 1,020 c.c. in the course of 96 hours.

One of the notable and important features of this organism is the large amount of acids which are formed during fermentation both of carbohydrates and proteins. At the same time the ammonia formed is sufficient to maintain the reaction at the initial level, or one even finds the fermented medium more alkaline than at the start. The reaction resulting from fermentation is never acid enough to destroy the organism.

Two other features of the growth action of this bacillus are worthy of notice. One is the putrid smell, the cause of which is not known. Volatile sulphides are formed, but these by no means account for the characteristic odour. The second feature is the rapid blackening which takes place in cooked meat. This is probably due to the action of the sulphides on the iron compounds present in the medium. The action can be developed more easily as Henry (1) has shown by the addition of carbonate of iron.

B. histolyticus. This organism, as the name implies, is supposed to be characterized by its vigorous proteolytic action. This is so, but it does not appear from the experiments which have been carried out that the proteolysis is more rapid or extensive than that obtained with *B. sporogenes*; neither is the gas production so vigorous.

A detailed investigation of the acid production has not been made, but it must be very great, for with a large production of ammonia, the reaction of the medium remains almost constant. The volatile acids formed in a concentrated cooked meat medium reach the high level of a 5 per cent. butyric acid solution.

One of the characteristics of *B. histolyticus* is the appearance in cooked meat medium of fine silky crystals. These have not been carefully examined, but they have the naked eye appearance of an amino acid like tyrosin.

B. oedematiens. This organism has a proteolytic action much less vigorous than that of the two preceding bacilli, but it attacks proteins more readily than either *B. welchii* or *vibrion septique*. Its gas forming power is not great, although in milk as much as

750 c.c. of gas may be formed per litre of medium. The volatile acid production is comparatively small.

Bacillus welchii. This organism is distinguished by its extraordinary power for gas formation. This is possibly best shown in a carbohydrate containing medium like milk. In a litre of this fluid as much as 3,800 c.c. of gas, consisting of carbon dioxide and hydrogen have been evolved in 20 hours. In a cooked meat mixture in which carbohydrates were not present 1,072 c.c. have been given off in 8 hours. This was an exceptionally vigorous fermentation, but 300 c.c. per litre in 10 hours is a very usual amount.

The carbohydrate consumption can be very large indeed and corresponds to the stormy fermentation in milk which is so characteristic of this organism. On one occasion 13·8 grams of lactose per litre of milk disappeared while 2,714 c.c. of gas were evolved from 1,000 c.c. of the medium. Even in a liquid like alkaline casein 344 c.c. of gas per litre have been given off.

The proteolytic action of the *B. welchii* cannot be disregarded. In media in which there is a large proportion of amino acids additional amounts are formed. In no case has a definite proteolysis been found wanting. Both ammonia and amino acids are produced.

The volatile acid production naturally varies with the composition of the medium, but in a nutrient mixture like cooked meat the acids formed are only $\frac{1}{4}$ to $\frac{1}{10}$ of what is found with *B. sporogenes.* This is undoubtedly due to the acids slowing down metabolism by reason of their hydrogen ion effect. Whether in a medium more strongly buffered an increased acid production can be effected is not known, but experiments are now being made in this direction. The practical point in connexion with the acid production of *B. welchii* is, that in cultures in a medium which has a resemblance to muscle, the acids formed are not of the order which one would expect to produce systemic effects.

The effect of hydrogen ion concentration on the growth of *B. welchii* has been examined, and it is found that metabolism ceases at an average $P_H = 4{\cdot}82$ in glucose peptone medium. The variation which takes place in the inhibiting concentrations of hydrogen ions due to individual acids are small. The principal volatile acid produced in metabolism is butyric acid.

Vibrion septique. This organism in its capacity for gas formation in a milk medium is in the same class with *B. welchii.* On one occasion 2,453 c.c. of gas were evolved from a litre of this fluid. The method of gas production is, however, quite distinct. While with *B. welchii* this amount of gas may be formed in 24 hours, *vibrion septique* will occupy 218 hours in doing so. The last 1,000 c.c. were formed in the particular experiment under discussion between the 168th and the 218th hours. This is certainly a ' late ' fermentation. The proteolytic activity is of the same order as that of *B. welchii.* It does not appear to form as much volatile acid as *B. welchii* under similar conditions.

Bacillus proteus. The biochemistry of this facultative anaerobe was examined because of the possible symbiotic effect it might have on the growth of true anaerobes. All the strains used were isolated

from wounds. None of the strains showed the putrefactive characteristics so often attributed to the micro-organism. It is a gas former, especially in meat media. Very moderate proteolysis was exhibited. No indol was produced. The volatile acid production was small. No notable difference was observed in the growth of cultures in the presence of air from those which were grown under anaerobic conditions.

6. Experimental Gas Gangrene.

A general account of the results, obtained by inoculating animals with broth cultures of wound anaerobes, has been given in the preceding pages. It is proposed in this section to deal with the more general problems in the pathology of gas gangrene and the light thrown upon them by experimental work; to show how far the accepted facts can be explained and whether the conceptions of the disease which have been formed as a result of general clinical observation and post-mortem experience can be verified by animal experimentation.

(i) *Infection.*

When a culture of a non-pathogenic anaerobe, such as *B. sporogenes*, is injected under the skin, as a general rule the animal remains in good health and is apparently unaffected. Examination of the site of inoculation, however, shows that a local effect, generally oedema and congestion, occurs. If films are made and examined, the organisms are seen undergoing extra-cellular lysis and phagocytosis. When the number of bacilli injected is very large the leucocytes emigrate in such quantities as to form what amounts to an abscess, which may lead to necrosis, but which is almost invariably followed by the healing process.

The same general results follow the inoculation of a saline suspension, free of toxin, of the pathogenic anaerobes.

It is evident, therefore, that the means by which the body protects itself against these microbes consists in lysis and phagocytosis and that the pathogenic organisms are of themselves non-virulent.

When a culture of one of the organisms which elaborate toxin is injected—that is to say, when the organisms and their metabolic products are injected together—the result is different. Lysis and phagocytosis do not occur; on the contrary the organisms multiply and invade the tissues, producing at the same time fresh toxins which the animal is unable to neutralize, and this process continues until death ensues.

A study of these facts has shown very clearly that the main, indeed all-important, difference between the pathogenic and non-pathogenic anaerobes of gas gangrene, from the point of view under consideration, is this capacity to form a toxin; and further that the toxins act as aggressins, that is to say, annul the animal defences and enable the microbes to proliferate freely in the tissues.

Toxin does not stand alone in this respect. Other substances have been discovered which are able to rupture the defensive system of an animal; their mode of action is different from that of toxin but it is not yet clearly understood. The first and probably the

most important of these substances are the ionizable calcium salts (Bullock and Cramer (1)). When washed and detoxicated bacilli of *B. welchii*, for example, are injected together with small doses of calcium chloride—2½ mg. for a mouse, 5 mg. for a guinea-pig—under the skin, the animal becomes ill in 8 to 10 hours and almost invariably succumbs within 24 hours to a violent gas gangrene.

Sterile distilled water in large doses—1 c.c. for a mouse of about 15 grm.—leads to the same result as do calcium salts, though not so regularly, nor yet with the same violence. Certain colloids show a similar behaviour when injected with the spores of *vibrion septique*. The most efficient colloid so far examined is silicic acid; the less efficient are gelatine, colloidal gold, palladium, and iron.

These facts, established during the war, call to mind the work of a number of investigators in which it is claimed that sand, lactic acid, cultures of staphylococci, and other materials may enable washed and heated spores of *B. tetani* or of *vibrion septique* to germinate in animal tissues and set up infection. It has not been possible to confirm these statements.

Such is the broad outline of the modes of infection in experimental gas gangrene. How far do they explain the occurrence of gas gangrene as observed during the war?

When a man is wounded and the wound becomes gangrenous it is clearly not sufficient to infer that this is because the wound is infected with a pathogenic anaerobe, since we may with safety conclude that toxin, beyond an infinitesimal amount, is never found in mud or soil. It is difficult to see why any wound should go beyond the stage already described for non-pathogenic and washed pathogenic anaerobes, namely a local gas abscess. The difficulty has been explained by the supposition that the nature of the wound is such that the infecting organism is able to grow and produce toxin. Thus, the presence of large masses of dead muscle and an impeded circulation, the condition of shock and depression of vitality are all factors which are supposed to explain this paradox, that non-virulent organisms may become violently virulent. These factors do undoubtedly play an important part in the gangrene process. Dead muscle is a good culture medium and in shock the circulation is defective and consequently resistance to bacteria is weak. Experiment has shown that cold may be an important factor in the evolution of the disease. But after giving due weight to all these facts and possibilities it cannot be said that they are sufficient to explain the occurrence of gas gangrene in the majority of cases. Experimentally it has not been possible to elicit with regularity a fatal gas gangrene in animals by imitating the conditions enumerated. Further, it is a matter of common observation that cases of fulminating gas gangrene sometimes occur in men in whom the amount of tissue destruction in the wound is small and the circulation is not noticeably impeded.

The minor and comparatively unimportant type of anaerobe infection known as a gas abscess, in which simple incision and free drainage are generally sufficient treatment, corresponds no doubt to the result obtained in animals when whole cultures of the non-pathogenic or suspensions of washed pathogenic anaerobes are

injected into the subcutaneous tissues, except that in the wounded man there is generally a sufficient quantity of necrotic muscle to provide a fermentable pabulum.

The fulminant cases of gas gangrene which occur in 6 to 72 hours after the infliction of the wound are similar in character to the experimental cases in which either a broth culture of the organism or calcium chloride and the organism have been injected. It is in these cases that cold, fatigue, and shock may play some part in the genesis of the disease; but the influence of the soluble calcium compounds in the soil contaminating the wound is probably of paramount importance.

It is a well-known fact that cultivated soil almost invariably contains soluble calcium compounds and that the quantity of these varies from time to time. They are produced by a complicated series of chemical reactions from insoluble calcium salts, notably the sulphate and phosphate, under the influence of bacterial activity, the sun and rain. It is, therefore, probable that the chemical composition of soil plays a large part in the production of this most tragic disease of war. For a long time it has been known that tetanus is liable to follow wounds contaminated with heavily manured soil. Here also it is now clear that it is not merely the presence of the tetanus bacillus, in no matter what numbers, which determines infection, but that other substances, of which calcium is possibly the chief one, enable the bacillus to live and multiply in animal tissues.

Whether calcium salts, silicic acid, and water, all constituents of soil, can reinforce each other in enabling organisms to break through the animal defences, has not yet been determined.

There is at present no reasonable explanation of the mechanism of infection in the rare cases of gas gangrene which occur, generally after secondary operation, weeks or months after the infliction of a wound.

A further point in connexion with the subject of infection is the steady fall in the proportion of cases of the disease as the war continued. It is a well-known fact and is discussed by Bowlby in the Hunterian Lecture for 1919. An altered condition of the soil, leading to a reduction in the numbers and a diminution of the virulence of the soil organisms, is one of the numerous causes brought forward to account for the decline. The alteration of the soil which is of direct consequence in this matter is probably the disappearance of calcium salts and of other similar substances, under the conditions described by Bowlby. It is less likely that the organisms lose virulence than that they decrease in numbers.

(ii) *The Established Disease.*

The general features of gas gangrene in men are observed in animals inoculated with broth cultures of the causative organisms. The local condition of oedema and gas production, the rapid invasion of the tissues by the bacilli, the occurrence of septicaemia, and the final toxaemia are all reproduced with striking fidelity. Any differences which exist are easily referable to anatomical or biological peculiarities of the animal employed. Thus, in mice, which are

very prone to septicaemia, *B. welchii* invades the blood stream an hour or two after a subcutaneous injection of a pure culture of this organism.

The striking feature of the disease which distinguishes it from almost all other infections is the rapidity with which it may lead to a fatal issue. It is not uncommon for death to occur within 24 hours after the active infection has begun.

It is indicated elsewhere in this Report that the toxins produced by *B. welchii* and *vibrion septique* are not very potent; a killing dose of less than 0·1 c.c. for a guinea-pig being rarely obtained. When such a toxin is compared with that of *B. tetani* in which the killing dose for the cavy may be as small as 0·0003 c.c., it is evident that the potency of the toxins poured into the tissues cannot account for the rapidity with which death may follow infection.[1] But in tetanus there is no general invasion of the tissues by the infecting organisms, whilst in gas gangrene this is a conspicuous phenomenon. It is probably safe to infer, therefore, that in the latter disease there is a greater multiplication of the pathogenic organisms and consequently a much greater amount of toxin elaborated. But this does not explain completely the extraordinary fact, that in a disease like tetanus in which the toxin is about a thousand times more lethal than that of *B. welchii*, death does not often occur sooner than 2 to 3 days after the infection is established, whilst in gas gangrene due to *B. welchii* death may occur in 12 to 18 hours.

The basis of these differences has been at least partly revealed. It has been shown (Bullock and Cramer (2)) that exhaustion of the suprarenal glands is of constant occurrence in animals killed either with sterile toxin or with cultures of *B. welchii* and *vibrion septique*. This can be most easily demonstrated in mice by means of Cramer's osmic acid vapour method. The suprarenals show congestion of the cortex and a great diminution of the cortical lipoids, while in the medulla there is a complete disappearance of adrenalin. Similar changes have been observed in actual cases of gas gangrene in men. It is the change in the medulla which is of special significance; a diminution of the cortical lipoid occurs in a number of septic conditions of diverse origin. The disappearance of adrenalin is not a temporary effect such as may occur after excessive stimulation of the gland, but the gland seems to be unable to re-form new adrenalin. The effect may be suitably described as an 'inhibition' or 'paralysis' of the organ.

The peculiar nature of the mode of death in gas gangrene consists in the fact that the toxins are assisted in their action on the adrenal bodies by a variety of factors, some of which are inherent in the infection, whilst others are adventitious to it and come into play only as the result of the special conditions obtaining in war wounds.

Thus it has been demonstrated experimentally that haemorrhage, the injection of acid, and exposure to cold lead to a disappearance of

[1] When small doses of the toxin of *vibrion septique* are injected intravenously in rabbits death may occur in a few minutes. But the same dose injected subcutaneously does not kill under approximately 24 hours, or may not even kill at all. The result obtained by intravenous inoculation is clearly not applicable to the disease, i. e. to the result of the infection.

adrenalin from the suprarenal medulla. It has also been shown that these three conditions transform doses of toxin which are non-lethal for a normal animal into lethal doses.

The bacteria of gas gangrene are very active acid producers and are assisted in this respect by other common wound organisms, such as *B. sporogenes*, which by themselves are not pathogenic. The production of acid at the site of injection—or in a wound—proceeds therefore concomitantly with that of toxin. Both are continuously being formed in rapidly increasing quantities as the local lesion extends, are absorbed into the circulation and assist each other in exhausting the suprarenal gland, which has already been largely depleted of adrenalin by such extraneous factors as cold and haemorrhage. The general conception of the infection which emerges from experimental work is therefore as follows.

The disease begins, not when a wound has become infected with the pathogenic anaerobes, but from the moment when a group of these bacteria have been enabled to surround themselves with a toxin sufficiently concentrated to abolish the local defences of the tissues. This condition may be brought about by one or more of the substances already enumerated which rupture the primary defences before toxin is produced. The organisms multiply rapidly and invade tissues which are often cut off from an active blood supply or are debilitated by the bacterial poisons. Acids are produced, and these promote the pullulation of the microbes (Wright and Fleming) and assist the systemic toxic effects by acting on the suprarenal bodies, which are also affected by shock, fatigue, cold, and haemorrhage. The consequences which flow from this ' paralysed ' state of the adrenals favour the invading microbes. A vicious circle is established which, unless rapidly broken, leads to that fulminant character of the disease which Wright has so aptly compared to the progress of an avalanche.

A general theory of gas gangrene which has found wide acceptance is that it is essentially a disease of muscle, that is to say, that muscle is always affected and generally contains the primary focus of infection, and that unless muscle is involved the classical type of the infection does not follow. This belief finds no support in experimental work. It is almost as easy to produce a fatal gas gangrene in animals by subcutaneous as by intramuscular inoculation. Further, when a culture of *vibrion septique* for example is injected intravenously into a rabbit, if the animal does not die within an hour of the effects of the toxin, it may die in 24 to 48 hours of an infection of *vibrion septique*. Post-mortem examination shows the bacilli distributed throughout the body but reveals no special predilection for muscle. It is possible also to produce the disease by intravenous injection of the organism deprived of its toxin. Thus if mice are injected intravenously with washed and heated spores of *vibrion septique* together with 0·7 mg. of silicic acid, they die within 24 hours and the bacillary forms of the organism can be seen in large numbers in the heart blood of the animals.

7. The Aerobic Infections of War Wounds.

By Alexander Fleming, F.R.C.S., Eng.

In the early stages of gunshot or shell wounds the association of anaerobic and aerobic bacteria is an almost constant one. The primary infection of these wounds, coming as it does from mud and material from the skin of the soldier, gives rise to a growth of anaerobic and of aerobic bacteria most of which, like the anaerobes, have a faecal origin.

Stokes and Tytler have carried out a series of observations on the bacteria found in recently inflicted wounds on their arrival at a casualty clearing station. In most cases examinations were made within twelve hours of the infliction of the wound, and it was found that out of 365 cultures, 310 showed the presence of aerobic bacteria. In cases where the species of aerobe was identified they obtained the following results :

Table I.

Aerobic bacteria found in wounds on admission to a casualty clearing station.

Organism.	*Number of cultures obtained.*	*Percentage.*
Haemolytic streptococcus	30	18·2
Non-haemolytic streptococcus	64	38·8
White staphylococcus	89	48·5
Yellow ,,	23	14
Tetragenus	22	13·3
Diphtheroids	12	7·2
Gram-negative cocci	15	9
,, ,, bacilli	38	23
Gram positive bacilli	59	35·7
Negative	23	13·9

Total number of cultures examined = 165.

These results should be contrasted with others, obtained in the later stages of wounds, at a base hospital in France and at a hospital in England (Tables II and III).

Table II.

Common types of bacteria, other than the spore-bearing anaerobic bacilli, found in wounds at a base hospital in France (Fleming (2)).

Time since infliction of wound.	*No. of wounds examined.*	*Strepto-cocci.*	*Staphylo-cocci.*	*Coliform bacilli.*	*Diphtheroid bacilli.*[1]
1 to 7 days	127	102	40	37	9
8 to 20 days	56	51	16	18	21
Over 20 days	27	24	19	19	16

[1] Some of these bacilli were obligate anaerobes.

TABLE III.

Common types of bacteria, other than the spore-bearing anaerobic bacilli, found in wounds in a hospital in England[1] *(Douglas, Fleming, and Colebrook).*

Organism.	*On admission to hospital.*	*At any period during their stay in hospital.*
Streptococcus . . .	48	53
Staphylococcus . . .	33	45
B. pyocyaneus . . .	8	23
B. proteus	19	29
B. coli type	19	29
Diphtheroid bacilli . .	34	43

Total number of wounds examined = 54.

The faecal element of the infection tends to disappear. The large anaerobic spore-bearing bacilli become fewer in numbers, and the streptococci which in the primary infection are usually of the non-haemolytic faecal type, become replaced by others which usually conform to the type of *S. pyogenes.* In a wound, therefore, after about two weeks when the sloughs have largely disappeared and when the 'healthy' suppuration has become established, the organisms usually found are streptococcus pyogenes, staphylococcus aureus, diphtheroid bacilli, and sometimes *B. proteus* and *B. pyocyaneus.* As these organisms are absent in most of the recently inflicted wounds, the question arises what is their source? There can be little doubt that in most cases they are hospital infections, and it is probable that they are spread from case to case in the dressing of the wounds, although sometimes, doubtless, the secondary invaders gain access to the wound from the skin around. On several occasions I have taken cultures from every patient in a ward and have found that only one or two were infected with *B. pyocyaneus* or *B. proteus,* but when I took cultures again from the same patients in the same ward a week or ten days later I found that every patient was infected with one or other of these organisms.

Again, as regards the haemolytic streptococcus (which in wound infections is practically always streptococcus pyogenes), this organism was found in only 18·2 per cent. of wounds on admission to a casualty clearing station (Table I). During the summer of 1918 Captain Porteous and myself examined a large number of wounds (compound fractures of the femur) on admission to No. 8 Stationary Hospital and found that only 20 per cent. of these were infected with haemolytic streptococci where the wounded had been sent immediately to the base after the primary surgical cleansing. In similar cases, however, which had remained in the same base hospital for more than a week, being dressed every day, it was found that over 90 per cent. were infected with haemolytic streptococci.

We see then that, whereas the primary infection of wounds is a mixed aerobic and anaerobic one, the secondary infection is almost wholly aerobic. This might be expected when it is remembered that the anaerobic bacilli of wounds will in general grow in a serous fluid only when the implantation is a large one, whereas

[1] These men remained in hospital in London for an average of something like six weeks, during which time examinations were made of the bacteria about once a week.

the aerobes will grow with much greater readiness, and especially streptococcus pyogenes which is by far the most common micro-organism of wounds in their later stages, and the one which persists after all the others have disappeared. According to Sir Almroth Wright's nomenclature, the anaerobic bacilli are of the nature of sero-saprophytes, i. e. possessing the power of readily growing out in serum only when that serum has been altered from the normal especially in regard to a lowering of its anti-tryptic power, while organisms like streptococcus and staphylococcus are serophytes, i. e. possessing the power of growing freely in unaltered serum. Sir Almroth Wright's work in this connexion has done much to explain the change in the bacterial flora of wounds.

Reference to Table I will show that on examination at a casualty clearing station almost 36 per cent. of wounds contained aerobic Gram-positive bacilli other than diphtheroids, and most of these were spore-bearing bacilli. In the cases I have examined on their arrival at the base I found about one in three to contain these bacilli, which appear to have no pathogenic power, and their importance to the bacteriologist lies mainly in their morphological resemblance to some of the important anaerobic bacilli. In the wound it is possible that they have some importance owing to their symbiotic action on the anaerobic bacteria. This question will be dealt with later.

(i) *B. mesentericus, B. subtilis, B. mycoides groups.*

These are the most common types of aerobic spore-bearing bacilli found. They are large, Gram-positive, and in a film made from a wound may resemble *B. welchii* or one of the other large anaerobic bacilli. Frequently very long elements may be seen or the bacilli may be arranged in chains. Central, subterminal, and terminal spores are to be seen, but in most cases the spores are not much broader than the bacillary body.

(ii) *B. aero-tetanoides.*

This bacillus resembles *B. tetani* morphologically but grows aerobically. It is motile and ciliated. It gives bluish colonies on agar with flowery prolongations. It is feebly proteolytic and does not ferment any sugar.

(iii) *B. aero-tertius.*

This bacillus I have recovered from wounds in cases admitted to a base hospital in France and also at a later stage in England. It bears a remarkable resemblance to *B. tertius*. It is Gram-positive, motile, and grows on ordinary media both aerobically and anaerobically. The colony on agar resembles that of *B. tertius* closely, being small and transparent with slightly irregular edges. It does not digest coagulated egg or serum, and its cultures have no putrid smell. It does not liquefy gelatin. It is a powerful saccharolytic organism fermenting glucose, lactose, saccharose, dextrin, starch, salicin, mannite, and glycerin, with the formation of acid and a small quantity of gas.

(iv) *Staphylococci.*

In the recently inflicted wound it is very common to find staphylococci of the albus variety while the aureus type is rare. These white staphylococci do not differ from the white staphylococci found in open wounds in civil practice.

Staphylococcus aureus is common in wounds in the later stages. It is very seldom found in pure culture, being almost invariably associated with streptococci and other organisms. A severe staphylococcus infection is always associated with much tissue necrosis. Occasionally it gives rise to a generalized infection. The incidence of *staphylococcus aureus* in war wounds seems to be very similar to its incidence in the open suppurating wounds which come into the casualty room of a London hospital.

(v) *Streptococci.*

This group is by far the most important of all the non-sporing bacteria found in wounds, and it is responsible for most of the deaths from sepsis in the later stages of wound infections. There are found in wounds two well-defined types of streptococci—the faecalis type, especially frequent in the recently inflicted wound, and the pyogenes type which is very common in the later stages. Other types occur but much more rarely.

Streptococcus faecalis. This organism is referred to in French literature as the ' enterocoque '. It is part of the primary infection of the wound and it gradually diminishes in numbers so that two weeks after the injury it cannot in most cases be discovered at all. It is present in the intestinal contents of man and animals and it is very commonly found in manured soil. Houston and McCloy recovered it on every occasion from mud scraped off the boots of wounded men on their admission to hospital.

Streptococcus faecalis is a large oval coccus generally occurring in pairs, the individual cocci being often set at an angle to each other. In culture it tends to be ' pleomorphic ', large and small, round and oval cocci being found. It grows well on all the ordinary media. On peptone agar the colony is a little, but not much, larger than that of *Streptococcus pyogenes,* but when it is planted on Douglas trypsin agar it grows with great luxuriance, the culture resembling staphylococcus rather than streptococcus. It ferments glucose, lactose, saccharose, mannite, and salicin but not raffinose or inulin. Usually it does not liquefy gelatin, but some strains have this power as also of digesting coagulated egg and serum. In broth it grows well, giving an even turbidity, in sharp contrast to the *Streptococcus pyogenes* culture in this medium.

This organism, although classed as an aerobe, prefers anaerobic conditions and sometimes when first isolated it will only grow in the absence of oxygen. In glucose broth under anaerobic conditions it grows very rapidly and luxuriantly and forms chains of twenty or more elements.

One of its most striking characters is its remarkable resistance to certain adverse circumstances. It lives for a very long time in culture. It will resist heating for half an hour at 55° C., and

sometimes will survive after being heated for 10 minutes at 80° C. Its growth is not inhibited by ox bile and it grows well on Drigalski Conradi medium. Houston and McCloy have used heating to 55° C. for half an hour as a means of isolating the organism, and Weissenbach has employed glucose peptone water to which has been added one-tenth of its volume of ox bile to differentiate this organism from *S. pyogenes*. In this medium *Streptococcus pyogenes* is completely inhibited while *Streptococcus faecalis* grows well.

Animals inoculated with a vaccine of this organism develop agglutinins to the strain with which they were inoculated but they do not of necessity develop agglutinins to all the other strains. The subject, however, requires further investigation.

Streptococcus pyogenes. In war wounds as in civil practice this is the most dangerous of all the pyogenic microbes met with, and it is the cause of nearly all the septicaemic conditions occurring in the later stages of wound infections. During the eight months from April to November 1918, working with Captain Porteous at No. 8 Stationary Hospital, we obtained forty-four positive streptococcal blood cultures and the streptococcus in every case belonged to this group. Of the other positive blood cultures which we obtained from wounded men, two were of *B. welchii*, one of *B. oedematiens*, and two of *Staphylococcus aureus*. These cultures were all made from cases of septic compound fractures of the femur and the results show the enormous preponderance of streptococcus pyogenes in generalized infections.

Source of the streptococcal infection. As we have seen (Table I), haemolytic streptococci are present only in 18·2 per cent. of the wounds soon after infliction. In the later stages nearly all the wounds contain this organism. It is usually a hospital infection. Levaditi and Delrez found that 54 per cent. of the English soldiers had streptococci habitually in the epithelial squames of the skin. Among the Belgian soldiers in rest, they found that only 12 per cent. had streptococcus in the skin although 62 per cent. showed the presence of this organism on the skin when they were coming out of the trenches. They state also that the wounds among the Belgians were less often contaminated with streptococci than those among the English. It may be then that the infection in some cases is by direct spread from the surrounding skin. It seems probable, however, that the infection is from case to case in the dressing of the wounds. It has long been known that *B. pyocyaneus* can easily spread from case to case in a ward unless the most rigid precautions are taken. The spread in this case is obvious to the surgeons owing to the colour imparted to the dressings, but unfortunately the streptococcal infection does not manifest itself to the naked eye until some disaster like a spreading cellulitis or a general infection results. In view of Sir Almroth Wright's finding, that of all the microbes found in wounds streptococci will grow most easily in the unaltered serum, it is probable that it is much more easily carried from case to case than *B. pyocyaneus*.

Characters of streptococcus pyogenes. In films of pus from wounds this organism occurs either in pairs, or in long chains, which are usually seen to be made up of pairs of cocci. Very frequently the

cocci are intracellular and when so placed can be seen in all stages of disintegration.

In young cultures the cocci are usually round and nearly uniform in size, but in older cultures they vary very much, and it is common to see one or two elements in a chain very much larger than the others. The individual cocci may become elongated, pear-shaped, or almost bacillary in old cultures.

Streptococcus pyogenes grows on all the ordinary media, and on Douglas trypsin agar forms rounded, grey colonies about 1 mm. in diameter with a very slightly irregular margin and a definite dark area in the centre when seen by transmitted light. In broth it grows in woolly masses which settle to the bottom or adhere to the sides of the tubes, leaving the supernatant fluid clear.

Growth occurs slowly on gelatin at 20° C. It does not liquefy gelatin or coagulated serum.

In milk it usually produces acid and a firm clot which later contracts and expresses a clear whey. The clotting of milk by *Streptococcus pyogenes* is, however, variable and appears to depend very much on the calibre of the tube used in the test. The smaller the tube used the quicker does clotting occur, and sometimes there will be a definite clot in a small tube when even after long incubation no clot will be produced in a large tube.

Sugar fermentations. The sugar reactions of this organism seem to be variable. Usually it produces acid in glucose, lactose, saccharose, and salicin, but not in mannite, raffinose, and inulin. A certain number of the strains (about 12 per cent.) ferment, in addition, mannite. Apart from this difference, the mannite fermenters and the non-mannite fermenters appear to be the same morphologically, culturally, and serologically.

Haemolytic power. All strains develop a haemolysin in culture. This haemolysin is very unstable, being rapidly destroyed by keeping or by heating to 60° C. McNee and Macleod showed that filtered broth cultures contained a powerful haemolysin. It was found impossible to produce an anti-haemolysin by the injection of the haemolysin into animals.

Viability. It usually dies out in a month or less on agar or in broth, but it can be kept alive for a long time without subculture on Dorset's egg medium or Robertson's meat medium.

Methods of isolation. This organism, like streptococcus faecalis, in general prefers anaerobic conditions, and a certain number of strains will only grow anaerobically when first isolated, although after one or two subcultures they become accustomed to aerobic conditions.

Usually an agar plate, preferably incubated anaerobically, suffices for the isolation of this streptococcus. It is often an advantage to use a blood-agar plate as the haemolytic action is then made manifest. The simplest method for this purpose is to pour a plate of ordinary agar and then after it has set to pour over the surface a thin layer of blood agar. Thus the haemolytic action is not obscured by the opacity of a thick layer of blood agar.

If *B. proteus* is present, isolation by simple plating may be difficult owing to the spreading growth of this microbe obscuring the

streptococcus colonies. In such a case cultures can readily be obtained by Wright's pyo-sero culture method or by growing anaerobically in glucose broth for six hours, diluting and then plating on agar or blood agar.

Blood culture methods. Douglas and Colebrook have shown that broth containing active trypsin is much better than ordinary or glucose broth. To 5 c.c. of broth 0·25 c.c. of trypsin (Allen and Hanbury's) is added and the tubes are incubated to ensure that they are sterile; 1 c.c. of blood is then added to each tube. In such a medium it has been found that growth occurs with a smaller implantation and is noticeable earlier than when plain broth is used.

Another method which has given very good results is to put 2 or 3 c.c. of blood into sufficient sterile distilled water to produce complete laking. This diluted blood clots slowly and there is plenty of time to carry it from the bedside to the laboratory and deal with it before clotting occurs. The laked blood is mixed with 15 c.c. of melted agar at 45° C. and a plate is poured. This method, in addition to giving merely a positive or negative result, gives an indication of the number of bacteria in the circulating blood. In a case of generalized streptococcal infection following a septic wound the number of streptococcus colonies obtained from 1 c.c. of blood is usually under 100 and often only one or two colonies develop. In one case, however, which was nearing a fatal issue, we obtained as many as 1,000 colonies from 0·25 c.c. of blood.

It has also been found that Robertson's meat medium, such as is used for the growth of anaerobes, gives better results than does plain broth in the cultivation of streptococci from the blood, and this medium has the advantage that not only will it yield a growth of streptococci which are indifferent to oxygen, but also of those which at first are strict anaerobes as well as the obligate anaerobic bacilli if they are present.

Serum reactions of Streptococcus pyogenes. It has been found that a rabbit injected with a vaccine of *Streptococcus pyogenes* develops agglutinins to this organism, and that while the serum obtained in this way agglutinated all the strains of *Streptococcus pyogenes* up to the same dilution, it did not agglutinate other streptococci except in a few cases, and then only in a very slight dilution.

A rabbit received first a dose of 1,000 million streptococci subcutaneously; four or five days later 1,500 millions were injected intravenously, and this was followed by three intravenous doses of 8,000 millions at intervals of one week. The rabbit was bled nine days after the last injection. The serum thus obtained was found to agglutinate emulsions of the homologous streptococcus up to a dilution of 1 in 500.

In performing agglutination tests with *Streptococcus pyogenes*, difficulty has been experienced in obtaining a good emulsion, and Douglas has devised the following method for growing fluid cultures of streptococci for this purpose. The medium consists of two parts of broth (Douglas') and one part of serum or hydrocoele fluid which has been heated to 60° C. for 30 minutes. The cultures were incubated in a slanting position. In this mixture the *Streptococcus pyogenes* was found to grow in such short chains

that on shaking up the culture an almost even turbidity was produced. The culture should then be diluted with 0·85 per cent. salt solution to a convenient strength. Twenty-four strains of *Streptococcus pyogenes* were found to be agglutinated by the serum of a rabbit inoculated as above with one strain, to the same titre (1 in 500). It was found that the strains which fermented mannite but which otherwise had the characters of *Streptococcus pyogenes*, were agglutinated exactly as were the non-mannite fermenting strains.

It appears from these experiments that the mannite fermenting and the non-mannite fermenting strains of *Streptococcus pyogenes* are essentially the same.

Anaerobic Streptococci. In 1915 I described an anaerobic streptococcus as being of frequent occurrence in wounds. It was found in 9 out of 12 wounds taken at random. Cottet found a true anaerobic streptococcus in 10 out of 33 wounds.

This streptococcus was difficult to isolate, as it always occurred in association with *Streptococcus pyogenes*, and the colonies of the two were identical. It grew in long chains and in old cultures showed very marked involution forms. It did not clot milk, and gave no change of colour on neutral red egg medium (*Streptococcus pyogenes* always gives a bright red colour on this medium). In shake or stab cultures on glucose agar, growth only occurred in the depths. Only one of my strains was carried through many generations, and it maintained its true anaerobic characters. The fact that these cocci did not give a red colour on neutral red egg medium indicates that they are a different type from *Streptococcus pyogenes*, which invariably gives a bright red colour. No serum reactions have been carried out in connexion with these cocci.

It is unnecessary here to go into the characters of the various other streptococci which have occasionally been found in wounds, as the work which has been done on them during the war has added little to our knowledge of them. It is interesting, however, to note that Malone and Rhea found that in penetrating chest wounds it was common to find streptococci of the type normally to be found in the respiratory tract.

(vi) *Diphtheroid Bacilli.*

Bacilli of this type are more common in the later stages of wound infections (see Tables I, II, and III). They have generally been regarded as of little importance and as leading merely a saprophytic existence.

True Diphtheria Bacilli. Fitzgerald and Robertson reported that out of 67 cases arriving in Toronto between May 20 and June 7, 1917, true diphtheria bacilli were recovered in 40. These bacilli were identified both by cultural and inoculation tests. In some cases, but not in all, the wounds showed the characteristic membrane of a diphtheritic infection. The extraordinary prevalence of this infection was partly explained by the fact that one of the nurses who was looking after these wounded men had a slight wound on her finger from which *B. diphtheriae* was isolated.

Fitzgerald and Robertson's report led to an investigation of the diphtheroid bacilli in wounds in some of the Canadian hospitals in England, and the results of this investigation have been published by Adami and others. Out of 306 cases investigated, bacilli were found in four which had the morphological and cultural characters of *B. diphtheriae*, and of these four, two were found to be pathogenic to animals producing the lesions characteristic of the Klebs-Loeffler bacillus.

Prior to this, in the latter part of 1916 and the early part of 1917, Douglas Fleming and Colebrook, in an investigation of wound infections at St. Mary's Hospital, found diphtheroid bacilli in 43 out of 54 cases at some period during their stay in hospital, and out of these 43 diphtheroid bacilli isolated, five morphologically and culturally appeared to be true diphtheria bacilli. These five strains were inoculated into guinea-pigs, and four of them were found to be virulent.

It seems clear, therefore, that a certain proportion of the wounds in England were infected with virulent diphtheria bacilli. In our series at St. Mary's Hospital none of the wounds showed any sign of a membrane, and they were all large flesh wounds healing up without any clinical sign of the presence of the bacillus.

Anaerobic Diphtheroid Bacilli. Some of the diphtheroid bacilli seen in films from wounds, especially in the early stages, are obligate anaerobes, and we isolated from wounds on a number of occasions a bacillus which we called the 'Wisp' bacillus, which belonged to this group. It was Gram-positive, non-motile, long and slender, and it arranged itself more or less in the typical diphtheroid manner. It grew anaerobically on agar or glucose agar in colonies slightly smaller and more transparent than those of *Streptococcus pyogenes*. We were unable to test its fermentative activities, and there seems to be no record of them elsewhere. Cultures on agar rapidly die out.

In wounds in the later stages it is very common to see large numbers of diphtheroid bacilli in films of the pus, but when this is planted out there is only a very scanty growth. It is possible that some of these bacilli, and especially those which can be seen cramming the leucocytes, are really acne bacilli which have gained access to the wound from the surrounding skin. The acne bacillus is one of the most common inhabitants of the skin, and in pus or in culture it shows a very definite diphtheroid arrangement. In pus from an acne pustule, also, it is very frequently found in large numbers inside the leucocytes. This bacillus only grows freely under anaerobic conditions when first isolated, and even then it does not appear in culture for three or four days, so that in the ordinary routine of wound examination where plates are made and incubated for 24 or 48 hours it would be missed altogether. The acne bacillus is to some extent a serophyte, and it is not unreasonable to assume that as it is so common on the skin it would sometimes gain access to a wound in the same way as staphylococci or *B. pyocyaneus*.

Aerobic Diphtheroid Bacilli. There is not complete agreement between different observers regarding the characters of the common diphtheroid bacilli found in wounds. At St. Mary's Hospital we

examined 34 strains, and found that they grouped themselves as follows:

Group.	*No. of Strains.*	*Characters.*
1	15	Large opaque white or creamy colonies on agar. Short stout septate bacilli. Only show a few Neisser's granules. Ferment glucose and saccharose with formation of acid. Do not ferment mannite, glycerin, or dextrin.
1 a	4	As group 1 except that the culture medium becomes a rich tawny brown colour in the presence of oxygen.
2	7	As group 1 except that they ferment mannite in addition. The mannite fermentation was always slow and there was no acid formation for two or three days.
3	3	Bacilli longer than group 1 and growth on agar not so copious. Do not ferment any of the sugars.
4	4	Bacilli have the morphological characters of *B. diphtheriae* with many Neisser's granules. Ferment glucose, glycerin, and dextrin with acid formation. Pathogenic for guinea-pigs, killing them with oedema at the site of inoculation and with enlarged and hyperaemic adrenals.
4 a	1	As group 4 except that they were not pathogenic for animals.

In this series the sugar tests were made in Cole and Onslow's broth, using acid fuchsin as an indicator. None of these bacilli formed acid from lactose with the exception of one strain in Group 4.

The Canadian observers, using Hiss's serum water medium, found that 16 strains out of 41 fermented lactose. They did not test the fermentative action on mannite. Their results are shown on Table IV.

TABLE IV.

Type.	*No.*	*Dextrose.*	*Lactose.*	*Saccharose.*	*Dextrin.*
Wound diphtheroid 1	6	A	A	A	A
,, ,, 2	6	A	A	A	0
B. diphtheriae (virulent)	2	A	A	0	A
,, (non-virulent)	2				
Wound diphtheroid 3 (xerosis type)	23	A	0	A	0
B. Hoffmann	2	0	0	0	0

A = acid formation.

The most common type of diphtheroid bacillus found in wounds, therefore, appears to be an organism of the xerosis type which grows luxuriantly on agar, and which ferments only glucose and saccharose.

The diphtheroid organisms generally have been regarded as being of little importance in wounds, and it has been found that wounds could be closed by secondary suture when the number of diphtheroids present was relatively high. Some of the infections with true virulent diphtheria bacilli have apparently resulted in the formation of the characteristic membrane, but this has not been a constant feature even when virulent diphtheria bacilli were isolated.

We shall see later that the non-pathogenic diphtheroids have a powerful symbiotic influence on the anaerobic bacilli and on streptococci, and in the connexion they may have some importance in the wound.

(vii) *Coliform Bacilli.*

In this group are included all the organisms which in a Gram-stained film resemble the *B. coli* type. Many varieties of these bacilli have been isolated from wounds in all stages, but usually it has been found that they are more common in the later periods than they are in the first few days after the wound is inflicted. Stokes and Tytler (Table I) found that 23 per cent. of the wounds on admission to the casualty clearing station contained these bacilli. Their incidence in the later stages can be gauged from the following table compiled from figures given by Stewart working in Leeds and Fleming working in Boulogne.

TABLE V.

Time after infection.	*No. of cases investigated.*		*Percentage incidence of coliform bacilli.*	
	Stewart.	*Fleming.*	*Stewart.*	*Fleming.*
Under 7 days . .	17	127	41	29
8 to 20 days . .	47	56	42	32
Over 20 days . .	58	27	74	70

From this table it is obvious that infection with these bacilli takes place very largely in hospital. It is very noticeable that in a wound there may be on one occasion many coliform bacilli, but when cultures are taken a week later they have disappeared, and later they may be replaced by a coliform bacillus of a different type. With few exceptions they seem to be merely passing saprophytes which do not really infect the wounds but which, probably due to some temporary favourable condition, gain access to the wound and proliferate in the discharges until such time as the condition which was favourable to their growth is changed. Some experiments by Wilson are very instructive in connexion with coliform infections. This observer planted a clean wound copiously with a living culture of *B. coli* from the intestine of the patient and then observed the fate of the organisms. He found that they had nearly all disappeared in 24 hours, and at the end of 48 hours they could not be seen in films or recovered in culture.

Two very definite types, *B. pyocyaneus* and *B. proteus*, occur frequently in wounds, but there are in addition a number of other types more or less definite which are met with. The classification of the coliform bacilli of wounds has been studied by Matthew J. Stewart, who isolated 148 strains from 122 wounds and arranged them as shown in Table VI.

TABLE VI.

Group.	*No. of Strains.*	*No. of Varieties.*	*Percentage Case incidence.*
B. coli	49	34	26
B. proteus . . .	29	4	24
B. Morgan, No. 1 .	7	2	5·7
B. faecalis alkaligenes .	1	1	0·8
Group X	8	3	5
Group Y	26	4	20
B. pyocyaneus . . .	24	1	20
Unclassified . . .	4	4	3

The chief characters which have determined the place of the organisms in the above table are given by Stewart.

B. coli. Fermentation of glucose and lactose with or without the formation of gas.

B. Morgan, No. 1. Fermentation of glucose, laevulose, and galactose only with the formation of acid and gas.
Formation of indol.
Voges and Proskauer's reaction absent.

Group X. Fermentation of glucose, saccharose, laevulose, galactose, and inosite with the formation of acid but no gas. Non-fermentation of lactose.
Litmus milk rendered acid and then strongly alkaline. No clotting.
Motility present.
Formation of indol.
Slow liquefaction of gelatin.
Voges and Proskauer's reaction absent.

Group Y. Fermentation of galactose without formation of gas.
Non-fermentation of laevulose.
Motility absent.
Indol negative.
Voges and Proskauer's reaction absent.

B. proteus. Fermentation of glucose and saccharose with the formation of acid and gas.
Non-fermentation of lactose.
Rapid liquefaction of gelatin.
Clotting and bleaching of litmus milk and finally more or less digestion of the clot.
No formation of indol.
Voges and Proskauer's reaction absent.

B. faecalis alkaligenes. Fermentation of none of the carbohydrates tested.
Motility present.
Litmus milk rendered strongly alkaline.
Gelatin not liquefied.
No formation of indol.
Voges and Proskauer's reaction absent.

It is unprofitable to discuss at length all these different varieties of coliform bacilli as they seem to play little part in wound infections. Stewart in his article deals with them fully. On account of their relative frequency, however, *B. pyocyaneus* and *B. proteus* deserve some further mention.

(viii) *B. pyocyaneus.*

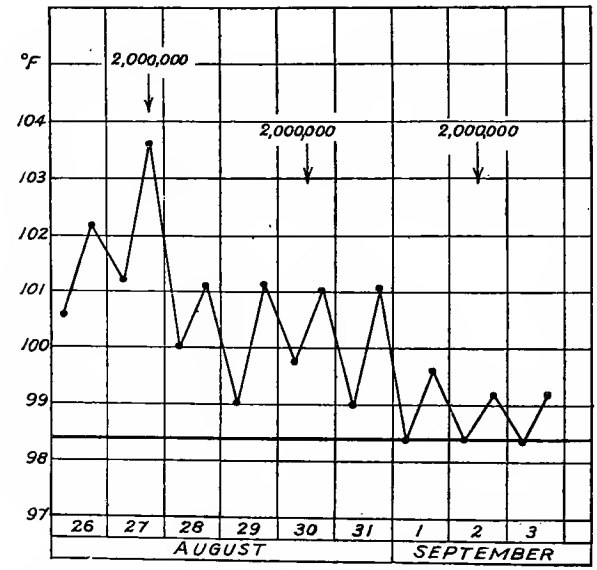
The war has not added much to our knowledge of this organism. The incidence of the bacillus in wounds varies much in different hospitals and in different wards of the same hospital, but in general it seems to be more frequent the longer the wounded man has remained in hospital.

The colour produced by different strains on agar may vary from almost jet-black to a very pale green. Some strains hardly produce any colour.

In some cases it seems to exercise a definite pathogenic effect, and in some cases a vaccine of this organism has had a remarkable effect in reducing the temperature of a patient whose wound had become infected. A chart showing this is appended.

(ix) *B. proteus.*

This organism, like *B. pyocyaneus,* seems to be in most cases a hospital infection. Its importance to the bacteriologist is greatly enhanced by the fact that it usually spreads rapidly over the surface of the culture medium, thus rendering the isolation of other organisms difficult. It can usually be isolated from the other organisms by planting the material into the water of condensation of an agar slope and incubating aerobically, when after 24 hours



B. proteus will have spread as a sheet of growth up to the top of the tube, from which situation pure cultures can be obtained. Anaerobically growth is much delayed, and if it is desired to isolate other organisms from *B. proteus,* anaerobic methods are of great assistance.

This organism rapidly liquefies gelatin, and it will digest coagulated serum or egg, but its proteolytic activities are not nearly so marked as those of a bacillus like *B. sporogenes.* Cultures have an unpleasant but not a putrid smell. In trypsinized serum considerable quantities of gas are evolved in the growth of *B. proteus.*

Sera of patients suffering from infections of this organism will frequently agglutinate this bacillus in some cases up to a dilution of 1 in 100 of the serum.

An animal inoculated with a vaccine of *B. proteus* develops agglutinins, and it was found that the serum of a rabbit inoculated with a vaccine of one strain agglutinated not only this strain but all the other strains to which it was tested (15 in all) up to a dilution

of 1 in 20,000 of the serum. Another rabbit, however, inoculated with another strain of *B. proteus* agglutinated the homologous strain to a dilution of 1 in 5,000, but only three of the other strains were agglutinated by this serum in a 1 in 1,000 dilution, and one out of the 15 strains was not agglutinated even in a 1 in 50 dilution. By means of absorption tests the existence of sub-groups of this organism can be demonstrated, but it appears that the differences between these are relatively slight.

Pathogenicity. In many cases this organism appeared to cause little trouble in wounds, but sometimes it seemed to be the actual cause of much or all of the fever from which the patients were suffering. It has been noted above that the patient's serum in many cases agglutinates the organism, which one would not expect if it were leading only a saprophytic existence. It has been observed that better results have been obtained in the vaccine treatment of wounds with a mixed vaccine of *B. proteus* and streptococcus than were got by streptococcus alone.

It is possible that the chief importance of *B. proteus* in a wound is due to its symbiotic action on the other bacteria.

(x) *Gram-negative Cocci.*

These are present in some of the wounds from the beginning (see Table I) and in the later stages they are occasionally to be found. Some of these Gram-negative cocci, and especially those found in the earlier stages of the wound, are strict anaerobes, and are similar to the Gram-negative cocci that can frequently be isolated from faeces.

One variety of Gram-negative coccus found in wounds is an obligate aerobe. It is somewhat larger than a staphylococcus, non-motile, and apart from a certain number of diplococcal forms, shows no special arrangement. It grows readily on agar in large white round colonies with a dull surface. It ferments glucose with the formation of acid but it has no action on any of the other sugars. It is not pathogenic for animals, and apparently exists in a wound merely as a saprophyte.

Wilson and Steer have made use of this organism for growing anaerobic bacilli in symbiosis without any other anaerobic precautions. As this coccus fermented glucose only, they were able to test the sugar reactions of the anaerobes by growing them in open test-tubes containing the sugar broth, which they implanted with a mixture of the anaerobe and this Gram-negative coccus.

(xi) *Micrococcus tetragenus.*

Cocci arranged in tetrads were very frequently seen in wounds, especially in the earlier stages. Some of these are obligate anaerobes which grow on agar or glucose agar in minute greyish colonies. These is no evidence to show that these tetrads seen in wounds are the same as the *Micrococcus tetragenus* of Gaffky.

Wound infections in the later period of the war.

Wound infections as seen at base hospitals were to some extent different in the later period of the war from what they were at the

beginning. In all probability this was due to the careful surgical cleansing which the wounds received at the casualty clearing station towards the end. In the early stages of the war the wounds came to the base containing large masses of slough in which all the faecal microbes which constituted the primary infection grew and flourished. In the later stages the infections seen at the base were much more limited to the pyogenic cocci and to the bacteria which gained access to the wounds at some period during the stay of the patients in hospital. In particular *B. proteus*, *B. pyocyaneus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and diphtheroid bacilli were frequently found.

As a result of the primary surgical cleansing the original infection was apparently much diminished and the conditions were not so favourable for its development, so that it disappeared rapidly except in cases where the primary excision had been incomplete and where considerable sloughs or pieces of necrosed bone had been allowed to remain in the wound.

8. Influence of the Aerobic on the Anaerobic Infection of Wounds.

By Alexander Fleming, F.R.C.S., Eng.

It is a function of the aerobic bacteria that they can, and prefer to make use of, the free oxygen dissolved in the medium and contained in the air above. This can easily be demonstrated by a very simple technique.

A tube of broth is planted with an aerobic organism. The cotton-wool plug is pushed a short way down the tube and is saturated with melted vaseline, after which melted vaseline is poured on the top of the plug to a depth of about one centimetre. The tube is incubated, and when the contents have become warmed to the temperature of the incubator the position of the plug is marked on the tube. It will be found that the vaseline is sucked down the tube owing to the absorption of oxygen during the growth of the bacteria. Thus, in a culture of a diphtheroid bacillus isolated from a wound, the plug was ultimately sucked down for such a distance that the volume remaining between the fluid and the vaseline plug was only four-fifths of the original volume. When pyrogallic acid and caustic soda were introduced into this tube there was no further reduction of volume and no blackening of the pyrogallic acid, showing that all the free oxygen had been taken up by the bacteria.

It can easily be imagined, therefore, that the aerobic bacteria may have a powerful effect in making the conditions in a wound suitable for the growth of the obligate anaerobes. Methods have long ago been devised for growing the anaerobic bacteria without special anaerobic apparatus in symbiosis with *B. subtilis* and many other aerobes, and recently Wilson and Steer have made use of an aerobic Gram-negative coccus for the same purpose.

The influence which aerobic bacteria have on the production of infection by spore-bearing anaerobes excited much attention even in pre-war days. Roger showed that an inactive strain of *vibrion septique* can be rendered pathogenic for a rabbit by injecting it in

association with *B. prodigiosus*. Vaillard and Rouget stated that tetanus spores would grow out in the tissues when they were injected with aerobic bacteria which protected them against phagocytosis, and a great deal of work has been done along these lines. It has been common experience during the war that an organism like *B. welchii* can produce a gaseous emphysema in animals in a much smaller dose if it is combined with some other organism, either aerobic or anaerobic.

Tissier ((3)–(6)) attributes the spread of the primary anaerobic infection in wounds to the action of the associated aerobic bacteria. He maintains that when the aerobes are harmless saprophytes the anaerobic actien is slow; it is more rapid when the associated bacteria belong to the pyogenic group; and it tends to become fulminating when the streptococcus is present. The aerobe not only favours the growth of the anaerobe, but it opens a way for it into the tissues. According to this author the commencement of the infection of the lymphatic channels is by streptococcus or staphylococcus.

Douglas, Fleming, and Colebrook (1) have studied the question of bacterial symbiosis *in vitro*, and they have found that all the common aerobes found in wounds have the power of stimulating the common anaerobes. Not only does the anaerobe grow more rapidly in association with the aerobe, but in some cases a growth was obtained with an implantation one million times smaller than when the anaerobe was grown alone. They found also that this stimulation of growth was shown as well, when the cultures were made under strict anaerobic conditions, as when semi-aerobic conditions obtained. The various anaerobic bacilli grown in association with *B. welchii* also exercised a beneficial effect on the growth of this organism and a symbiotic effect was observed in some cases when the different aerobic bacteria were grown together.

A few typical experiments will indicate the extent of this symbiotic action.

(i) *Influence of Staphylococci and Streptococci on the Growth of* B. welchii *in Milk.*

A broth culture of *B. welchii* was diluted by tenfold steps up to one in a million. 40 c.mm. of each dilution were planted into tubes containing (*a*) milk 1 c.c., (*b*) milk 1 c.c.+staphylococcus broth culture 20 c.mm. and (*c*) milk 1 c.c.+streptococcus broth culture 20 c.mm. After 24 hours the tubes were examined and the growth of *B. welchii* was noted by the formation of the typical clot. The result was as follows:

Micro-organisms planted.	*Dilutions of* B. welchii *culture.*					
	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,000
B. welchii only . .	GR	GR	0	0	0	0
„ staphylococcus	GR	GR	GR	GR	GR	GR
„ streptococcus	GR	GR	GR	GR	GR	GR

GR = growth of *B. welchii*. 0 = no growth of *B. welchii*.

(ii) *Influence of Aerobic Organisms on the Growth of* B. welchii *in Serum neutralized with Acid, and in Serum the antitryptic power of which has been neutralized with Trypsin.*

In this experiment miniature test-tubes were used, and after the implantations had been made, melted vaseline was poured on the surface of the culture fluid to make a column about 1 centimetre in depth. The index of growth of *B. welchii* was gas formation evidenced by the vaseline plug being pushed up the tube.

Dilutions of a broth culture of *B. welchii* were made as in experiment 1 and 10 c.mm. of each of these dilutions was added to 300 c.mm. of serum. The same procedure was repeated with serum infected with each of the aerobic organisms. The results obtained were as follows :

organisms planted.	*Dilutions of* B. welchii *culture.*						
	1/1	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,0(
hii only . .	GR	0	0	0	0	0	0
, diphtheroid	GR	GR	GR	GR	GR	GR	0
, streptococcus	GR	GR	GR	GR	0	0	0
, coliform bac.	GR	GR	GR	GR	GR	GR	GR
, *B. pyocyaneus*	GR	GR	GR	GR	GR	0	0

The same results were obtained when the alkaline reaction of the serum was neutralized with acid or when its antitryptic power was neutralized with trypsin. Sir Almroth Wright has shown that both of these procedures materially aid the growth of *B. welchii* in serum, but it appears from this experiment that the symbiotic action of the aerobic organism has nothing to do with its effect on the reaction or the antitryptic power of the serum.

(iii) *Influence of Aerobes on the Growth of Anaerobes other than* B. welchii.

(*a*) The technique employed was the same as in experiment 2. After 5 days' incubation the results were :

organisms planted.	*Dilutions of Broth Culture of* B. sporogenes.						
	1/1	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,00(
rogenes only	0	0	0	0	0	0	0
„ + streptococcus	GR	GR	GR	GR	GR	GR	GR

GR = growth of *B. sporogenes.*

(*b*) Equal volumes of serum were implanted with *B. sporogenes* and *B. tertius*. One tube of each was kept as a control, and to the others were added some diphtheroid bacilli staphylococci or streptococci. Melted vaseline was now run on to the surface of the fluid and the tubes were incubated. The results after 3 and 5 days were as follows :

Associated Micro-organisms.	*B. sporogenes.*		*B. tertius.*	
	3 *days.*	5 *days.*	3 *days.*	5 *days.*
None . . .	0	0	0	Growth
Diphtheroid bacillus .	Growth	Growth	Growth	Growth
Streptococcus faecalis .	„	„	„	„
Staphylococcus . .	„	„	„	„

(iv) *Influence of* B. welchii *on the Growth of Streptococcus and Staphylococcus.*

(*a*) Two tubes were put up, one containing 1 c.c. of serum planted with 10 c.mm. of streptococcus emulsion, and the other containing 1 c.c. of serum planted, in addition with 40 c.mm. of a *B. welchii* broth culture. After eighteen hours' growth under anaerobic conditions the number of streptococci were estimated by Wright's method. The result was as follows :

Tube containing streptococcus only =100,000,000 streptococci per c.c.
Tube containing streptococcus and *B. welchii*=360,000,000 streptococci per c.c.

(*b*) Four tubes, each containing 1 c.c. of serum were taken. Two of them were planted with *B. welchii* and all four were incubated anaerobically for twenty-four hours. An equal quantity of staphylococcus was then planted into one of the previously unplanted tubes and into one of the *B. welchii* cultures. The other two tubes received an equal implantation of streptococcus. After a further twenty-four hours' incubation aerobically the number of cocci was estimated by plating on agar 10 c.mm. of a 1,000-fold dilution of the culture. The number of colonies of the cocci which resulted was as follows :

Tube 1. *B. welchii* and staphylococcus gave about 600 colonies.
Tube 2. Staphylococcus only gave 64 colonies.
Tube 3. *B. welchii* and streptococcus gave about 1,000 colonies.
Tube 4. Streptococcus only gave 1 colony.

These two experiments show clearly that *B. welchii* stimulates the growth of streptococcus and staphylococcus.

(v) *Effect of a Diphtheroid Bacillus on the Growth of Streptococcus pyogenes.*

Into each of three small test-tubes 1 c.c. of serum was placed. To the first and second were added 10 c.mm. of a 1,000-fold diluted broth culture of streptococcus, while to the second and third were added 10 c.mm. of a 1,000-fold diluted broth culture of a diphtheroid bacillus. The second tube, therefore, had a mixed infection of these two organisms. The number of living microbes was estimated by plating out a known quantity on agar and counting the colonies. The tubes were then incubated for ten hours at 37 C. and the number of living bacteria was again estimated in the same way. The results obtained were as follows :

Implantation.	*Content of living Streptococci per c.c.*		*Content of living Diphtheroid bacilli per c.c.*	
	At time of planting.	*After 10 hours' incubation.*	*At time of planting.*	*After 10 hours' incubation.*
Streptococcus . .	13,000	2,800,000	—	—
Streptococcus and diphtheroid . .	13,000	120,000,000	2,000	2,600,000
Diphtheroid . .	—	—	2,000	5,300,000

This indicates that while the diphtheroid bacillus has a powerful stimulant effect on the streptococcus the action is not reciprocal.

It seems clear, therefore, from the work of many observers both *in vitro* and *in vivo*, that the aerobic organisms such as are found in septic war wounds have a powerful influence in stimulating the growth of the anaerobic spore-bearing bacilli, and this is manifested not only by an increased rate of growth of the anaerobe, but also by the fact that in association with the aerobic bacteria the anaerobes will flourish in a serous medium or will infect an animal in a very much smaller dose. In gas gangrene the anaerobe has been compared to the high explosive and the aerobe to the detonator. Bullock and Cramer have shown that other ' detonators ' exist in the shape of calcium and other salts, but it seems clear that the aerobic bacteria must not be forgotten in this respect.

It has been held that this stimulating effect is due to the aerobe absorbing oxygen and so rendering the conditions more favourable for the anaerobe, but although this may in some cases be an important factor it cannot explain the whole of the symbiotic effect observed. The stimulant effect of the aerobe is manifest whether the cultivations are made under perfect anaerobic conditions or whether the oxygen is not rigidly excluded. It has also been shown that the presence of a second anaerobe in a culture will stimulate the growth of *B. welchii*, and if mixtures of anaerobes are injected into animals, the lethal dose is very much diminished, and here there can be no question of the absorption of oxygen.

9. Résumé of the Literature on the Bacteriology of Gas Gangrene, with an Account of the Incidence of the Various Types of Pathogenic Anaerobes.

When cases of gas gangrene began to develop with such remarkable frequency shortly after the outbreak of war, bacteriologists found it necessary to take stock of their knowledge of this group of infections and, it must be confessed, found it largely wanting. Many workers had neglected to apply the tedious methods of technique which was necessary for the study of the few cases which came in the ordinary routine of work and their knowledge was admittedly second-hand and based upon the experience of certain ' classical ' predecessors.

It was understood that gas gangrene was not an aetiological entity but a syndrome capable of being produced by several bacterial species. Among these were the *vibrion septique* of Pasteur proved by Brieger and Ehrlich to be a cause of disease in man, *B. aerogenes capsulatus* of Welch and Nuttall and *B. phlegmonis emphysematosae* of Eugen Fraenkel. Further, it was clear from a study of the literature that a large number of bacteria had been described and named by other observers, which had no very marked points of difference, and the suspicion was prevalent that several of these new varieties were really identical and that the points of difference were illusory.

There can be little doubt that such confusion as existed was due in the first place to the difficulties of ' plating out ' anaerobic bacteria upon solid media and thus obtaining perfectly pure cultures, and secondly to the technique of ' enriching ' the anaerobic flora of a specimen by growth in ' selective ' fluid media thus causing some variety, perhaps an unimportant one, to assume an undue prominence

After the outbreak of war, a greater familiarity with the disease and improvements in technique led to an increase of knowledge which in France and Great Britain, if not in Germany, tended to become more stabilized. The pronounced characters of *B. welchii* and the ease with which it can be isolated, naturally led it to a position of prominence although disguised under different names. Fleming, Weinberg, Eugen Fraenkel, Simonds, Henry, and many others found the bacillus in practically all wounds or articles of clothing examined.

Much confusion has been associated with Pasteur's *vibrion septique* and Koch's *B. oedematis maligni*. Early in the war *B. sporogenes* Metchnikoff was found by many workers such as Robertson, Dean, Goadby, and Lardennois and Baumel. This microbe, a moderately large, motile bacillus with a central or subterminal spore possesses the power of liquefying serum and digesting milk. Such an organism had been described by Jensen and Sand, Kitasato (1), Kerry, Silberschmidt, Theobald Smith, v. Hibler, v. Werdt, and others as *B. oedematis maligni* of Koch which itself was assumed to be identical with Pasteur's *vibrion septique*. Long ago, however, Sanfelice (1892) pointed out that an organism which produced the typical lesions of Pasteur's experimental disease was not putrefactive and proteolytic as was then being taught. During the war Weinberg and Séguin, McIntosh, Sacquépée, and others began to report the presence of a motile, non-proteolytic bacillus in war wounds and from its pathogenic and morphological characters identified it with Pasteur's *vibrion septique*. An extended study of this microbe has confirmed this view and has shown that it differs entirely from the proteolytic *B. sporogenes*, the assumption being that proteolytic cultures of *B. oedematis maligni* are not pure. In order to clear the confusion as to the real relationship of these non-proteolytic pathogenic organisms a short account of their history is given.

Unfortunately, neither Pasteur nor Koch left any complete biological or cultural description whereby the bacilli called by them *vibrion septique* and *B. oedematis maligni* respectively, could be subsequently identified with certainty. In every instance the source of their virus was the animal cadaver. In modern bacteriological text-books it is usually stated that *B. oedematis maligni* can be readily obtained by inoculating guinea-pigs with garden soil. The accuracy of this statement must, however, be questioned. A great deal of the confusion which exists at the present time as to what is and what is not *vibrion septique* of Pasteur or *B. oedematis maligni* of Koch, is probably due to this statement. The majority of the writers who followed upon Koch seem to have been content to regard as the bacillus of malignant oedema any sporing anaerobe obtained from the lesion caused by inoculating soil into animals, with the result that a considerable variety of characters has been assigned to this bacillus. Sanfelice and C. O. Jensen, however, state that the bacillus of malignant oedema can only be obtained from garden soil with difficulty, while Passini never found it there.

Von Hibler (1) in 1899 described three strains of malignant oedema which all liquefied serum; later, in his monograph of 1908, he reaffirms that his strains of this bacillus all liquefied serum and

digested milk, as did those of Kitasato and Silberschmidt. The type considered to be the bacillus of malignant oedema by Theobald Smith and by C. O. Jensen, digested milk. Divergences of opinion are to be found in regard to the morphology of the bacillus, some describing the spore as more or less terminal (Roux, Chauveau and Arloing), others that it is chiefly central (Jensen and v. Hibler). There is also no agreement as to the fermentation reactions. Theobald Smith states that it may ferment glucose, lactose, and saccharose; Jungano and Distaso consider that glucose and lactose are fermented but not saccharose, while Bahr found that strains collected from different sources showed varying fermentations.

In consequence of these researches, characters were attributed to *B. oedematis maligni* (Koch) which we now know to belong to *B. sporogenes*, an entirely different organism. The confusion between these two bacterial types is perpetuated even in some of the most modern German writings on the anaerobic bacteria of war wounds.

In their elaborate and careful research Ghon and Sachs, assuming that the bacillus of malignant oedema was proteolytic, and finding that the organism which they had isolated was non-proteolytic, described it as a new bacillus of malignant oedema. There can, however, be little doubt now that the bacillus of Ghon and Sachs was the *vibrion septique* of Pasteur. Grassberger and Schattenfroh were also of this opinion, but Ghon and Sachs could not agree with them because they believed that the malignant oedema bacillus of Koch was proteolytic as described, and they further accepted Koch's statement that the bacillus of malignant oedema was identical with *vibrion septique*.

So far as we can ascertain at this distance of time the micro-organisms which were studied by Pasteur and Koch were identical; they were both highly pathogenic for laboratory animals; the lesions produced were identical, and smear preparations from the peritoneal surface of the liver of animals which had succumbed to the infection showed the characteristic long thread-forms from which the name *vibrion septique* was derived.

To resume the history of these researches during the war, certain workers by the use of selective methods have succeeded in demonstrating the existence of some particular anaerobe and concluded therefrom that the anaerobe in question was one of the principal causes of gas gangrene. In this category may be placed the view of Eugen Fraenkel with reference to *B. phlegmonis emphysematodes* (*B. welchii*), of Conradi and Bieling with their *B. sarcemphysematodes*, of Weinberg with *B. oedematiens* and of Sacquépée with *B. bellonensis*.

A certain unanimity was, however, reached by French and British bacteriologists and the following tables indicate the incidence of the respective types found:

Weinberg's results, 91 cases of gas gangrene.

B. perfringens (*B. welchii*)	77%
B oedematiens	34
B. sporogenes	27
B. fallax	16·5
Vibrion septique	13

Sacquépée's results.

B. perfringens (*B. welchii*)	82%
Vibrion septique typical	28
Vibrion septique atypical	11
B. bellonensis	35

McIntosh's results. Series A. 1914–18, 41 cases.

B. welchii	43·9%
B. sporogenes (including type XIII of McIntosh)	36·5
Vibrion septique	19·5
Terminal oval sporing bacilli	17·0
Unidentified types :	
Type No. XVIII	4·8
Type No. XIX	4·8

Series B. 1918. 52 cases.

B. welchii	67·3%
B. sporogenes (including type XIII)	38·7
Vibrion septique	16·3
B. oedematiens	4·0
Terminal oval sporing bacilli	8·1
B. tetanoides	2·0

Henry's results. 1917. 100 cases.

B. welchii	66%
B. sporogenes	48
B. tertius	22

Henry's results. 1918. 50 cases.

B. welchii	80%
Vibrion septique	16
B. oedematiens	10
Other pathogenic anaerobes, of which 6% are probably *B. fallax*	10

This last unpublished series of Henry's was derived from material obtained from cases of gas gangrene, sent to the Committee by corresponding members and medical officers serving with the British forces in France. It will be seen at once that the results differed from those which he obtained in 1917, but this can be explained by the fact that in his second series an entirely different method of analysis was employed the technical details of which were as follows :

A series of preliminary experiments had shown conclusively that there was no antagonism between *B. welchii*, *vibrion septique*, and *B. oedematiens*, if these were introduced together into an alkaline meat medium kept under suitable anaerobic conditions. For example, no evidence was obtained that *B. oedematiens*, reputed to give feeble growths, could be overgrown in culture either by *vibrion septique* or by *B. welchii*. Each of the three organisms mentioned, either alone or in association with the others, reached its full development in 12 to 18 hours when grown in a meat medium.

Pieces of infected muscle from the cases of gas gangrene were placed in tubes of meat medium, and the resulting cultures, 18 to 24 hours old, were used for inoculation into guinea-pigs. This ' whole culture ' method appeared to have certain outstanding merits :

(*a*) The transference of the micro-organisms from man to the guinea-pig occupied a very short time, and the loss of virulence was reduced to a minimum.

(*b*) Any advantage of microbic association would presumably be at a maximum in a mixed culture.

Of the 50 specimens of muscle, 44 yielded cultures which were pathogenic to 250 grm. guinea-pigs in doses of 1 c.c., death resulting in 12 to 24 hours.

A potent serum containing antitoxins to *B. tetani*, *B. welchii*, and *vibrion septique* protected guinea-pigs against 33 of these pathogenic mixed cultures.

In 5 out of 11 cases in which the triple serum yielded no protection, the infecting organism proved to be *B. oedematiens*, and the infection could be arrested with an anti-oedematiens serum. Of the remaining 6 cases 5 yielded anaerobes which were not *B. welchii*, *vibrion septique*, or *B. oedematiens*. Three of these five were probably pathogenic strains of *B. fallax*.

A glance at the above tables of results is sufficient to show that there is a considerable degree of uniformity in the findings of the British and French workers. The German results, however, are neither uniform nor do they agree with the above. To judge from the number of polemical articles which have appeared in the German medical press no finality has been reached, and it is apparent that this must be attributed to the fact that they have not yet succeeded in isolating the more important anaerobic bacteria in pure culture.

Aschoff (3) considered that there were three main types which he called :

1. ' Gasbrand Group '.
2. ' Rauschbrand group '.
3. ' Malignant oedema group '.

and these were again subdivided into pathogenic and non-pathogenic types :

	Non-pathogenic types.	Pathogenic types.
1. Gasbrand	*B. saccharo-butyricus immobilis*	Welch-Fraenkel type.
2. Rauschbrand	*B. amylobacter* *B. saccharo-butyricus mobilis* *B. paraputrificus*	Conradi-Bieling type, Ghon-Sachs ' bacillus, vibrion septique Pasteur, Kolmar type.
3. Malignant oedema	*B. putrificus*	Von Hibler's malignant oedema bacillus. Koch's malignant oedema bacillus ?

In a later paper, Aschoff (4) republished the table with slight modifications in that the ' Gasbrand group ' was called ' immobile butyricus group ' ; ' Rauschbrand group ' being called the ' mobile butyricus group ' while the ' malignant oedema group ' was renamed ' putrificus group '.

R. Pfeiffer and Bessau came to the conclusion that there were four main types :

A. *Non-putrefying anaerobes.*
   1. Bacillus of Fraenkel.
   2. Bacillus of malignant oedema (Koch).

B. *Putrefying anaerobes.*
   1. ' Uhrzeiger ' or clock-hand bacillus.
   2. Par-oedema bacillus.

Conradi and Bieling, however, considered that gas gangrene was due to one anaerobe, *B. sarcemphysematodes*, and that the Welch-Fraenkel bacillus, the bacillus of malignant oedema, the bacillus of Rauschbrand, &c., were merely different stages of *B. sarcemphysematodes*. Two main types were fairly constant, viz. type A, which is a plump, non-motile rod, producing in milk an acid clot, which is later digested, as is coagulated serum; type B which is thinner, actively motile and affects milk and coagulated serum in a manner similar to type A. They further state that type A can be changed into type B by a few passages on coagulated serum but type B can only with difficulty be changed into type A. They also claim that specific immune sera can be produced for each type. Conradi and Beiling have evidently taken their ideas from Grassberger and Schattenfroh's conception of the mutability of certain anaerobes, but as in the case of these earlier workers the evidence produced is unconvincing. The work of Conradi and Bieling received a considerable amount of confirmation, but other German bacteriologists, Pfeiffer and Bessau, however, strongly opposed their view as they had not found any evidence in its favour.

Klose (6) states that gas gangrene cannot be considered as due to any single species but must be ascribed to the action of a number of different bacilli. By serological tests he was able to sub-divide the anaerobes isolated, into four types.

1. Welch-Fraenkel group.
2. Putrificus group.
3. Rauschbrand group.
4. K_1 group (corresponding to Novy's bacillus).

An analysis of 100 strains isolated from soldiers wounded at Verdun showed the following grouping:

	per cent.
Group 1	34
,, 2	24
,, 3	32
,, 4 (K_1)	6
Unclassified	4

Of these strains 36 were obtained by venepuncture.

As regards these German results the position is not clear, and the discrepancies between the findings of individual workers are considerable. The descriptions of the biological and cultural characteristic which have been given do not enable us to identify with certainty the several species with which they were working. Their cultures have not been available for study in this country; it is, therefore, impossible to dogmatize, but reading between the lines

it is possible to suggest an interpretation of the significance of their various names as follows:

The Welch-Fraenkel group of the German workers can obviously be identified as *B. welchii.*

The Rauschbrand group of Aschoff and of Klose presents greater difficulty. The true Rauschbrand bacillus (*B. chauvoei*) has not been isolated from war wounds by any British or French worker. Klose, however, states that in the early days of the war it was found that the Rauschbrand serum, prepared at the Hoechst works, agglutinated a considerable number of the strains of anaerobes isolated from wounds. It was, therefore, concluded that they were Rauschbrand bacilli. Later, he tested 12 Rauschbrand cultures of animal, as well as of human, origin and found them to be identical in their agglutinating reactions.

The description of these so-called Rauschbrand types by Klose, Zeissler (3), &c., shows that they failed to differentiate the organism from *vibrion septique* of Pasteur. This failure possibly arose from the fact that the Rauschbrand serum in question (Hoechst) was prepared not by the injection of *B. chauvoei* but from strains of *vibrion septique* which had been isolated from animals. It has been conclusively demonstrated by several writers (Markoff, Koeves, Meyer, &c.) that *vibrion septique* may infect cattle, horses, and pigs with the production of symptoms similar to those of blackquarter. The supposition that Rauschbrand serum made at the Hoechst works was specific and might thus be used for differentiating *B. chauvoei* from other pathogenic anaerobes was not upheld by Pfeiffer and Bessau who stated that the clinical concept of 'Rauschbrand' is not an aetiological entity. Further, H. Landau in a series of agglutination tests showed that the Hoechst Rauschbrand serum did not agglutinate two strains (A and B) of *B. chauvoei* isolated by Kitt one of the classical authorities on the disease in question. A serum prepared from Kitt's strain B did not agglutinate any of the 'Rauschbrand strains' said to have been obtained from cases of human gas gangrene. In the uncertain state of present knowledge it is not possible to dogmatize, but the Committee consider that the existence of *B. chauvoei* in human gas gangrene is not proven.

The Putrificus group. The members of this group are proteolytic, liquefying coagulated serum and digesting milk. The pathogenicity apparently varies to a considerable extent. The 'Uhrzeiger' or clock-hand bacillus of Pfeiffer and Bessau would be included here. In the present state of our knowledge of anaerobic bacteria there is little doubt that the putrificus group corresponds to the sporogenes group of British and French workers. The fact that the German bacteriologists found a certain number of the strains to be pathogenic, a result not observed elsewhere, is explicable on the assumption that their cultures were contaminated with some of the pathogenic anaerobes. It is only this pathogenic effect which distinguishes the putrificus group from that of *B. sporogenes.*

The close unanimity arrived at by the British and French workers has been recorded above. The only discordant result is to be found in the work of Sacquépée (11) who attaches great importance to an organism which he calls *B. bellonensis.* From the

descriptions which he has so far published this organism cannot be identified.

In conclusion, the Committee are of opinion that acute gas gangrene may result from at least three types of pathogenic anaerobic bacilli; these in their order of frequency are as follows:

1. *B. welchii.*
2. *Vibrion septique.*
3. *B. oedematiens.*

In addition to these, many other anaerobes may be present in wounds. The exact part which these play in the production of gas gangrene is not clear. In pure culture they cannot be regarded as pathogenic for laboratory animals. The more common forms of these are:

B. sporogenes.
B. parasporogenes.
B. tertius (Hibler IX).
B. histolyticus.
B. fallax.

10. The Isolation of Anaerobic Organisms of Wounds in Pure Culture.

In the isolation of anaerobic bacteria no single method has been found which will meet every requirement. It cannot be too strongly insisted upon, that, whatever technique may be employed, pure cultures can be obtained only by the exercise of great technical skill and by the constant application of a critical attention.

(i) *Material Suitable for Examination.*

1. Exudates from wounds.
2. Pieces of infected muscle removed at operation.
3. Fluid from haemorrhagic bullae.
4. Blood from a vein.
5. Post-mortem material.

In the collection of the above material certain precautions are necessary. It is preferable that the bacteriologist should collect the material himself, so that it should be as little contaminated as possible. A sufficiently large sample should be taken to inoculate several tubes of medium. Further, a Gram-stained smear preparation of the wound exudate or muscle juice should be made, as this is frequently found to give useful indications of the varieties of anaerobes present.

(ii) *Methods of Isolation.*

Whatever the ultimate procedure adopted, the primary aim is to preserve all the anaerobes present in the sample taken, and for this, some medium as little selective as possible should be used. The alkaline meat medium or broth with a piece of fresh tissue are both useful for this purpose, and part of the material should be inoculated into one or other of these media, which may be regarded as a repository. Surface growths on serum agar under very good

conditions of anaerobiosis may be made direct from the wound material if this is derived from a relatively uncontaminated source. The early mixed cultures obtained in the meat or tissue medium tubes should also be planted in series on agar slopes or plates and the colonies picked off.

In addition to the above, a number of methods which are essentially selective in character have been found to be of great utility.

They comprise :

A. The separation by mechanical methods of the individual organisms in a mixture.
B. The selection by appropriate heating of the spores contained in a mixture.
C. The use of a selective media.
D. The separation of a pathogenic anaerobe by animal experiment.

A. *Separation by Mechanical Methods of the Actual Individual Organisms Present in a Mixture.*

Since the memorable discovery of Pasteur a great deal of attention has been paid to the cultivation of anaerobic bacteria but it is certain that prior to the war a large number of anaerobic species had not been obtained in a pure state. It is easy by some and possibly by all of the innumerable methods, which have burdened bacteriological literature, to cause anaerobes to multiply but few of the apparatus although mostly described as ' simple and effective ' will lead to the production of pure, isolated colonies on the surface of a solid nutrient medium—a result obtained with ease in the case of aerobes. Some of the factors which militate against success with anaerobes have been repeatedly emphasized in various parts of this Report. Of these, cohesion of the material leading to a close association of two or more anaerobes in one and the same colony is perhaps the most important and it must be further added that progressive dilutions may not necessarily overcome it. The high degree of motility of many anaerobes will also cause them to spread as a film over the surface obscuring others which may themselves have a predilection to assume isolated colonial form. This is especially prevalent in the presence of excessive moisture. Sometimes the medium is not suited to the requirements of all the several bacteria in a mixture and the spores of some may lie dormant on the surface and may be subsequently picked up along with a well-developed colony and continue with it in subsequent subculture. These are fundamental difficulties which must be expected and mastered if possible. The cohesion of the material may be overcome to a very considerable extent, as pointed out by Stoddard (1) if the original material and all the subsequent dilutions are thoroughly shaken with glass beads, sea sand, or even saline solution, surface cultures being then planted out. A medium which will encourage the growth of as many of the anaerobes as possible should be employed.

However interesting may be the fact that anaerobes can grow under apparently aerobic circumstances it is certain that separate colonies on solid media can only be obtained under conditions in

which oxygen is intentionally excluded and for surface growths the exclusion must be as complete as possible.

The exclusion or removal of free oxygen can be carried out in a number of different ways each having a set of supporters. The general principles involved are :

1. Cultivation *in vacuo*.

2. Deep cultures as recommended originally by Hesse and Liborius and subsequently developed by Veillon and Zuber and by Burri.

3. Cultivation in indifferent gases of which hydrogen and nitrogen are the chief.

4. Absorption of oxygen by chemicals such as alkali in the presence of pyrogallol, or in atmospheres deoxygenated by combustion with hydrogen in the presence of palladium as originally suggested by Laidlaw and carried to perfection by Fildes and McIntosh and Smillie.

5. Combined methods in which evacuation in the presence of alkaline pyrogallol, or hydrogen in combination with alkaline pyrogallol with or without evacuation are the chief.

Previous boiling of the medium—historically the oldest method—should be effected as far as possible but is inadmissible with many of the modern media containing coagulable protein.

For some reason, imperfectly understood at present, anaerobes often show a great disinclination to grow as separate surface colonies and but few of the many methods will lead to such a development. It is quite evident that for such surface growths the anaerobiosis must be much more complete than in the case where colonies are embedded in the medium. It would also seem to be proved by the researches of Burri and Kürsteiner that the anaerobiotic condition should be induced as rapidly and as completely as possible when once the anaerobes are implanted on the artificial nutrient medium.

The exact technical details of the various anaerobic apparatus should be consulted in the various periodicals and journals devoted to bacteriological literature. It suffices to say that in their extended and fruitful researches Weinberg and Séguin employed deep cultures after the manner of Veillon and Zuber, Henry utilized, in particular, special dishes rendered anaerobic by alkaline pyrogallol while Miss Muriel Robertson relied principally on thorough removal of oxygen by the air pump. McIntosh was unusually successful with a simple apparatus in which the air was deoxygenated by hydrogen in the presence of palladium asbestos. In most cases it is found that test tubes are preferable to actual Petri plates for isolation of surface colonies.

A microbic emulsion may be diluted down to a point at which a minute volume sufficiently small to be examined microscopically can be shown to contain only one individual organism. Each minute fraction of the emulsion which contains a solitary organism will if planted out into an appropriate medium give rise to a culture which is pure because it is derived from one individual. This method of obtaining a pure culture has been practised by a number of workers in the isolation of aerobes, and it has been applied by several investigators in the case of the anaerobes. The larger size of most anaerobe bacilli, as compared with that of aerobic organisms,

a feature which greatly facilitates a microscopic count, appeared at first sight to be a factor which would enhance the chance of success. It was found, however, that of the solitary bacilli isolated from a mixture of anaerobes only a very small percentage proved to be viable. This difficulty, taken in conjunction with the other well-known disadvantages that are inherent in any micro-inoculum technique, renders the method inapplicable to the isolation of anaerobes on an extensive scale. There are, however, occasions when the procedure in capable hands may prove to be of very great value. (Barber.)

B. *The Selection by Appropriate Heating of the Spores Contained in a Mixture.*

This method which has frequently proved to be very serviceable in the isolation of anaerobes has been practised with two objects in view.

1. To separate spores from vegetative forms.

A small volume of a mixed culture sealed off in the capillary portion of a Pasteur pipette, is plunged into boiling water for fifteen seconds or subjected to a temperature of 80° C. for twenty minutes by immersion in a water bath. This heating is sufficient to kill off all vegetative forms so that only spores remain. By this means there can be removed completely from a mixed culture (*a*) non-sporing aerobes such as streptococci, *B. proteus* and coliform organisms, and (*b*) non-sporing anaerobes. The latter include *B. welchii*, *B. fallax* and *B. aerofetidus* when the mixture has been grown in a carbohydrate-containing medium such as milk, glucose broth, &c.

The heated portion of culture is inoculated into a liquid medium and the resulting subculture is used for plating out.

2. To separate the more highly resistant spores in a mixture from those that are less resistant to heat.

Von Hibler (3) practised this method and claimed good results for it.

The use of heat to destroy non-sporing organisms is of proved value. On the other hand, we have not at the present time enough reliable evidence to determine the utility of the method when it is applied to the differential separation of one species of spore from another. The almost ubiquitous appearance in cultures of *B. sporogenes* the spores of which are particularly resistant to heat would appear to militate against success.

C. *Selective Media.*

If a mixed growth of anaerobes in a tube containing meat medium be examined at repeated intervals, its bacterial population will be found to alter from day to day. The organisms which are the first to develop include such types as *B. welchii*, *B. fallax* and *vibrion septique*, all of which reach their maximal growth within twenty-four hours of inoculation. After twenty-four hours, these organisms are replaced by others of a totally different character, such as *B. sporogenes* ; and this second developmental phase in the culture is succeeded by a third phase, in which end-sporers, of which *B. tetani* is the most important example, predominate.

The history of such a culture may thus be said to comprise three different periods or epochs, each of which is characterized by its own distinctive flora. The organisms of the first period have been found to be predominantly saccharolytic in character, while those of the second period are proteolytic. In the third period the medium is found to have reached a condition in which it is unfavourable to the growth of organisms of the first and second periods, but in which it is pre-eminently suitable for certain organisms of the end-sporing group.

Under favourable conditions the same sequence of events can be traced in the bacteriological history of a wound infected with anaerobes.

The information yielded by further investigation of the facts outlined above has proved of great assistance to the bacteriologist, for on it there is based the whole principle that controls and determines the choice of selective or differential media in the isolation of anaerobes.

(A) *Selective media for the saccharolytic anaerobes.*

The first stage in the isolation of members of this group of anaerobes consists in producing from the original tissue broth or meat culture a subculture which will contain an aggregation of individuals which display some particular biochemical feature. For example, an anaerobe which has the capacity to ferment a particular carbohydrate will grow rapidly in a medium containing that sugar and will be found to outnumber all the organisms that do not ferment it. It is to be noted, however, that this numerical preponderance may be in evidence only at a certain stage in the history of the culture. There are, therefore, two factors to be borne in mind, viz., (1) the presence of a fermentable carbohydrate which will ensure the development of organisms with the capacity to ferment that carbohydrate, and (2) the period in the history of the culture at which the numerical superiority in special individuals exists.

To ensure a further concentration of these individuals, all that is necessary is to make, in tubes of the same special medium, several subcultures in series, each separated from the preceding parent culture by an appropriate interval.

The second stage in isolation consists in making surface growths from the last subculture so as to get separate colonies which can be picked off and grown again in the selective medium. The agar employed for this purpose should be mixed with serum or alkaline egg medium. Where plain agar only is available, the admixture of the material to be inoculated with serum or alkaline egg before spreading it on the agar surface gives very good results. The agar may be used in Petri dishes or as slopes in test tubes, according to the choice of the individual worker. Many of the anaerobes tend to give continuous films of growth on a moist surface and it is therefore desirable to free the medium from condensation water as far as possible. The deep agar shake method which has been practised by many workers, particularly by the French, gives results that are distinctly inferior to the surface growth method. It is, however, a useful method when surface growths cannot be obtained.

It is to be noted, that of the many methods which have been devised for obtaining anaerobiosis in the case of surface growths on solid media, only a very few provide conditions sufficiently perfect to promote development of the more strict anaerobes. The method devised and described by McIntosh and Fildes (1) has been tested extensively by a number of workers during the war and has been found to give excellent surface growths. The use of a metal or glass cylinder from which the air can be extracted by means of a pump and replaced by an atmosphere of hydrogen, the process being repeated two or three times to ensure the absence of oxygen, provides a method which has been severely tested by Miss Robertson and others at the Lister Institute with equally good results.

It is of great advantage to examine surface colonies with a pocket lens or under a dissecting microscope. It is only by this means that one can distinguish differences between colonies that look alike to the naked eye.

A colony which appears to be isolated may be found to be surrounded by many others, or it may be seen to have developed in the midst of a continuous surface film, both of which conditions are difficult to detect by the unaided eye. The examination may also reveal the growth of a contaminating organism in a culture presumed to be pure. The distinctive appearance in texture and outline shown by colonies of certain of the anaerobes has been described in that section of this Report which deals with the bacteriological features of the individual organisms.

As specific instances of the application of selective methods in the isolation of carbohydrate-fermenting organisms, the following examples may be cited.

1. *B. welchii.*

B. welchii attains its maximal development in freshly boiled glucose broth tubes in three to six hours. It is thus possible to pass the same material through two tubes between the morning and evening of the same day. The second culture is plated out in the evening and left to grow overnight. Colonies picked off the next morning may again be passed through glucose broth. The alternation of growth in glucose broth combined with plating out can be repeated as often as is necessary. It has been found that a growth which is reasonably certain to be pure can be obtained in six days, i. e., after six agar platings and twelve rapid passages through glucose broth.

Maltose would seem to be more rapidly and more vigorously fermented by *B. welchii* than is glucose, so that with a 1 per cent. maltose broth or casein digest medium, it is possible to make from four to six serial replants in the course of a day.

2. *Vibrion septique.*

This organism ferments salicin. The reaction takes longer to develop than does the fermentation of glucose or maltose by *B. welchii* and does not reach its maximum till twelve to twenty-four hours after inoculation. A medium containing salicin may, therefore, be used to concentrate *vibrion septique* where it is associated with *B. welchii* and *B. sporogenes* for neither of the latter are capable of attacking salicin. The growth of such a microbic mixture in 1 per

cent. salicin broth or casein digest for twenty-four hours followed by plating on to agar containing 1 per cent. salicin, the process being repeated several times, has been found to yield a good chance of obtaining *vibrion septique* free from other organisms.

In both of the instances just quoted organisms other than those specially indicated are likely to be met with. For example, in the isolation of *B. welchii* by rapid passage through glucose or maltose broth tubes, *B. fallax* or *B. aerofetidus* may be found. Each of these organisms gives rise to colonies which after a little experience can be easily distinguished from those formed by *B. welchii*. Similarly the salicin method gives a good chance of obtaining *B. tertius*, but here again the colony can be readily differentiated from that of *vibrion septique*.

(B) *Selective media for the proteolytic anaerobes.*

The best known member of this group is *B. sporogenes*. It grows much more slowly than do the saccharolytic anaerobes. If a protein containing medium which is sugar free be inoculated with a mixture of anaerobes which includes *B. sporogenes*, it will be found that this organism outgrows the others. A medium made with tap water or saline containing bits of coagulated egg white offers no attraction to any anaerobe other than *B. sporogenes*. Small pieces of fish muscle or of crab muscle may be substituted for the hard boiled egg. An alkaline egg medium is also serviceable in obtaining a concentration of *B. sporogenes*. Material taken from a culture of two days or more is plated on to agar. After forty-eight hours' incubation colonies are picked off and sown into another tube of sugar free medium. As before, the process is repeated several times, until the organism appears to exist in pure culture.

(C) *Exhausted media.*

A medium in which *B. sporogenes* has grown for some time is found to be suitable for the development of *B. tetani*, *B. tetanomorphus* and *B. cochlearius*, but not for the development of the saccharolytic anaerobes or of *B. sporogenes* itself. It would seem to be selective for the organisms mentioned, and was used by Tulloch for this purpose.

D. *Separation of a Pathogenic Anaerobe by Animal Experiment.*

Guinea-pigs or mice may be used for this purpose.

1. *In normal animals.*

Where a mixture of organisms contains only one pathogenic anaerobe, then this particular organism can be recovered after death from the heart blood of an animal which has succumbed to an intramuscular inoculation of the mixture. If the pathogenic anaerobe be *vibrion septique* or *B. oedematiens* it may be obtained in pure culture from the animal's heart blood. It is to be noted, however, that certain strains of *B. oedematiens* may kill by intoxication without spreading into the circulating blood stream. Where the pathogenic anaerobe is *B. welchii* there is often a tendency for other organisms such as *B. sporogenes* or streptococci or coliforms to appear in the blood along with *B. welchii*.

On the other hand, where two or all three of these pathogenic anaerobes are present in a mixture, any one of them or any combination of them may be found in cultures made from heart blood.

It follows, therefore, that the use of a normal animal for such a passage experiment is not likely to give a reliable result unless one can be reasonably certain that only one pathogenic anaerobe is present in the inoculated mixture.

2. *In protected animals.*

A guinea-pig which has been passively immunized by inoculation with antitoxic sera against *B. welchii, vibrion septique* or *B. oedematiens* is capable of successfully resisting infection by the corresponding organism. For example, an animal adequately protected against *B. welchii* will never be found to develop a fatal *B. welchii* infection though it may readily succumb to *vibrion septique* or to *B. oedematiens* if either of these be present in the inoculum. The same holds good for *vibrion septique* and *B. oedematiens* antitoxic sera, the immunized animal being afforded absolute protection against infection by the corresponding organism but not against other pathogenic anaerobic organisms.

Further, it has been possible by using appropriate mixtures of these antitoxic sera to protect animals against a combination of any two or of all three of these organisms.

One may thus construct in a guinea-pig a sort of filtering mechanism which will retain or hold up certain organisms by inhibiting their development in the animal, and which will at the same time allow the growth and subsequent passage into the blood-stream of any pathogenic anaerobe against which no specific protection has been induced. This method was actually used in determining the pathogenic anaerobes present in specimens of muscle taken from fifty cases of acute gas gangrene in France. A combined serum containing 4,750 units of welchii antitoxin and 5,000 units of septique antitoxin per 10 c.c. was given intraperitoneally in a dose of 4 to 5 c.c. to guinea-pigs of 250 grm. weight, twenty-four hours before the inoculation of the injecting dose of mixed culture. The latter consisted in each case of 1 c.c. of culture mixed with an equal volume of the same serum and left in contact with it for one hour at room temperature before intramuscular inoculation. Of the protected guinea-pigs which succumbed none died with a *B. welchii* or *vibrion septique* infection. The organisms which were recovered from the heart blood of these animals proved in every case to be *B. oedematiens* or some other different anaerobe. The full results will be discussed in detail elsewhere. This animal filtration method has been given an extensive trial and it can be safely recommended as providing a reliable means of isolating from a mixture of anaerobes a pure culture of *B. welchii, vibrion septique* or *B. oedematiens*.

In a case of urgency, three guinea-pigs or mice protected with appropriate combinations of the three antisera (welchii, vibrion septique and oedematiens) may be inoculated with the wound discharge direct from the patient. A diagnosis of the pathogenic anaerobes present may be afforded by the result obtained in the animals over night.

E. *Summary.*

The above outline of the several methods which may be employed in the isolation of the anaerobes must be taken as affording no more than general indications in regard to the methods that are most likely to yield success. The individual researcher will develop them along the lines of his own particular need and will find that they are capable of modification and adaptation in a number of useful directions. An active and resourceful manipulation of the methods of isolation available is rendered necessary by the great variety in the problems presented.

The worker who sets out to obtain pure cultures of the anaerobes essays no easy task. There are fortunate occasions when he may be able to plant out the original material directly on to plates, as, for example, when he is dealing with a post-mortem heart blood or with pieces of infected muscle taken at some distance from a wound. In the majority of instances, however, he is faced with material that is grossly contaminated, and in dealing with it the following points should be constantly borne in mind.

1. Growth in a special selective medium is merely preparatory to and must alternate with plating out on serum agar. The anaerobic conditions employed must be rigorous enough to give good surface growth of the more exacting anaerobes.

2. The criterion of purity of a culture is based entirely on its consistent behaviour when it is grown on a large range of different media and when its cultural reactions are observed over a long period of time.

3. The worker need never hesitate to admit the impurity of one of his cultures when definite proof of the same is produced before him, for the very best bacteriologists have been deceived by the anaerobes. It is only by the exercise of constant vigilance and the maintenance of a keen critical attention in regard to the possibility of contamination that he can escape these pitfalls.

(iii) *Preservation of Cultures.*

A few general directions are given for the preservation of cultures when isolated.

The desirable conditions under which to keep stock cultures are those in which the organism will form spores which will retain their vitality over long periods. As a general rule, media containing fermentable sugar and milk media should be avoided as the acid reaction produced by many anaerobes is unsuitable for the prolonged maintenance of the culture.

The vast majority of the anaerobes may be subcultured on to meat medium, coagulated serum, or broth with a piece of coagulated egg white, and the tubes sealed in a blow-pipe after forty-eight to seventy-two hours' growth. The sealed cultures can be stored in the dark at the temperature of the laboratory for more than a year without losing their vitality. When the sealed tubes are opened they should be subcultured on to meat medium, a large inoculum being used. The strain should then be grown on milk, meat medium, coagulated serum and gelatine, and the sugar reactions tested so as

to assure the worker that the culture is pure and typical. It is often at this period when a resting culture is once more subcultured after a long interval, that contaminations are found in a supposedly pure culture.

Certain anaerobes require special treatment.

B. welchii is best preserved in alkaline egg fluid or on coagulated serum as spores are more certainly formed in these media than in the others in use.

B. fallax is an organism which is apt to die out in stock cultures, spores are not formed readily and it is not always a prolific grower. It is best kept in alkaline meat medium or coagulated serum and it should be incubated for at least three days before the tubes are sealed. Cultures should be made in triplicate and the strain should be subcultured every four to six months.

B. cochlearius strains are also easily lost. Meat medium of an alkaline reaction, broth with a piece of living tissue or coagulated egg white, are the best media for stock cultures. In pure culture this organism is not a prolific grower.

IV. SEROLOGY.

1. Toxins and Antitoxins.

(i) *Historical Introduction.*

A. *B. welchii.*

Since the appearance in 1892 of the classical article by Welch and Nuttall on the gas bacillus, the study of the pathology of infection by this organism has attracted the attention of many workers. The exact significance of the pathological changes that are induced in the animal body as the result of infection have been variously interpreted.

Eugen Fraenkel (1) looked upon the disease as being an intoxication due to the absorption of decomposition products formed in the tissues. Much the same view has been advanced in the course of the war by Conradi and Bieling (1) who considered that the various acids produced by the fermentation of carbohydrates were responsible for the tissue necrosis, and that this necrosed tissue favoured the development of saprophytic anaerobes, the resulting putrefactive products when absorbed giving rise to intoxication. Kamen in 1904 found that 8-day old cultures contained a haemolytic substance which could be readily demonstrated *in vitro* and decided that although strong toxic substances could not be produced *in vitro* they were probably formed in the animal body.

Passini (1) found that the toxic substances in filtrates from old cultures survived a temperature of 100° C. for 15 minutes, and further, that they possessed no antigenic properties.

McCampbell in 1909 attributed the lesions produced in animals and in man by culture filtrates to the presence of butyric acid. He found that lesions identical with those following the inoculation of filtrates could be produced by butyric acid alone and that the neutralization of filtrates rendered them inactive.

Stewart and West in 1916 came to the same conclusion. Of others who have investigated the toxic substances present in filtrates of *B. welchii* mention may be made of Korentchevsky, Herter, Simonds (1), Costa, and Troisier (3), and Ouranoff.

In none of the papers cited, however, is there any adequate demonstration that the toxic substances referred to possessed antigenic properties.

Rosenthal (1) appears to have been one of the first to attempt the preparation of a serum against *B. welchii*. He inoculated horses with gradually increasing doses of culture. The resulting antiserum proved to have only slight protective properties.

In 1916 Weinberg (6) reported that he had been successful in preparing an antibacterial serum to *B. perfringens*, the horse being immunized with washed bacilli. This serum, in a dose of 0·005 c.c., neutralized one lethal dose of culture in guinea-pigs. There can be little doubt that this serum was really antitoxic but the difficulties experienced by Weinberg in obtaining a good toxin derived from *B. welchii* militated against any successful demonstration by him of its antitoxic value. The first clear exposition of *B. welchii* toxin is to be found in the communications of Bull and Pritchett (1). These workers proved that the necrosing and haemolytic properties of *B. welchii* filtrates were due to the presence of a real exotoxin, which could be destroyed by heating to between 60° and 70° C., and which was capable of stimulating the production of antitoxin when inoculated into animals. They further showed that this antitoxic serum when employed under proper conditions inhibited and arrested infection with *B. welchii*.

B. *Vibrion septique.*

In contradistinction to our knowledge of *B. welchii* antitoxin, which is very recent, our information in regard to immunization against *vibrion septique* infection dates back to the publication of a communication by Roux and Chamberland in 1887, Duenschmann 1894, Leclainche and Morel 1901, M. Nicolle, Césari and Raphael 1915, Raphael and Frasey may all be cited as yielding proof of the production of protective sera against Pasteur's organism.

C. *B. oedematiens.*

This organism was described for the first time in the course of the war by Weinberg and Séguin ((1) (2)). These observers have accurately defined the properties of the specific toxin, and have succeeded in producing high-value antitoxic sera in horses. There is no proof that the organism described in 1894 by Novy as *B. oedematis maligni II*, and later by Migula as *B. Novyi*, produced toxin although Novy (p. 224) suggested that this was likely from the scarcity of bacilli in the oedematous areas.

(ii) *The Preparation of Toxin.*

A. *Toxin of B. welchii.*

Bull (1) produced *B. welchii* toxin by growing the organism in glucose broth containing large amounts of sterile rabbit muscle. The aseptic removal of muscle from a rabbit presents considerable

echnical difficulties. To eliminate this particular complication has been the aim of all those who have since dealt with the problem, and more particularly perhaps of those workers who have had to prepare toxin on a sufficiently large scale for the immunization of horses.

The preparation of toxin of *B. welchii* is beset with even more difficulties than is the production of the toxins of diphtheria and tetanus. There are so many variable factors to be considered in the process and many of these factors are so difficult of control that the issue can never be accepted as a certainty. With repeated experiment, however, and a fuller understanding of the essentials to success it becomes possible to establish the *in vitro* conditions which assure the probability of a fortunate result.

The points which appear to be of special significance may be considered under the following headings: (*a*) the medium; (*b*) the organism; (*c*) the inoculum; (*d*) the conditions of growth; (*e*) filtration.

(*a*) *The Medium.*—Experience has shown that the best medium for use on a large scale is a muscle broth which is prepared as follows:

1. A mixture of minced horse muscle and tap water, in the proportion of 1 lb. muscle to 1 litre water, with 0·5% common salt, is boiled in a cauldron for 1 hour.

2. The cooked meat is then removed and, to the broth, after filtration there is added 1 per cent. of peptone and 0·2 per cent. of glucose.

3. The broth is then heated till the peptone is dissolved, and sufficient caustic soda is added to make it slightly alkaline to litmus.

4. It is then boiled for half an hour and filtered to remove the precipitate.

5. Into a series of 4-litre bottles (double Winchester quarts) there are placed about 2 handfuls of the cooked meat and 4 litres of broth.

6. The bottles are plugged with cotton wool and autoclaved for 3 hours at a pressure of 22 lb. per square inch.

7. The end reaction of medium so prepared varies from 3 per cent. to 4 per cent. acidity when tested with phenol phthalein as the indicator.

Peptone appears to be a necessary constituent for the formation of toxin but it is to be noted that the commercial brands differ considerably in value.

Glucose has a powerful influence in promoting the toxicity of a filtrate, although its action in this respect must not be looked on as specific. It has a direct effect in encouraging the growth of *B. welchii* and an indirect beneficial effect in that after fermentation it provides the degree of acidity (phenol phthalein titration) that appears to be essential to the formation of toxin.

Reaction.—The end reaction of a *B. welchii* filtrate depends to a large extent on the initial content of the medium in fermentable carbohydrate. Bull was the first to demonstrate this clearly and the statement has been fully borne out by the findings of other workers. Starting with a sugar free medium Bull showed that the end reaction taken at intervals from the first to the thirteenth day of inoculation

lay between 2 per cent. and 2·5 per cent. acidity. By increasing the amounts of glucose in the muscle broth he found that the acidity rose, until, with a content of 3 per cent. in glucose an acidity was reached of 8 per cent. He further demonstrated that, although the presence of glucose in amounts up to 1 per cent. had little unfavourable influence on the toxicity of the filtrate, yet glucose in large amounts had a decidedly deleterious effect on toxin production. There is thus a distinct relationship between the end reaction of a filtrate and its toxicity.

Experiments conducted with 0·2 per cent. glucose muscle broth media in which the initial reaction has been arranged so as to vary from 3 per cent. acid on the one hand to 3 per cent. alkali on the other, have suggested that although the end reaction (after the growth of *B. welchii*) comes out at practically the same figure, viz. an acidity of 4 per cent. to 5 per cent., yet toxin production is most marked in media which were originally acid. It would seem therefore that *B. welchii* prefers an acid medium for the production of toxin and that if the organism be grown in an alkaline medium it expends its energies on the fermentation of the glucose present at the expense of toxin formation. Experiments dealing with this matter are still in progress.

The buffering of a medium with sodium phosphate has not, up to the present, materially influenced the production of toxin.

Fresh muscle.—The influence of fresh muscle in encouraging toxin formation is unquestionable. It has not been found necessary, however, to use the large volumes recommended by Bull. A small piece of muscle about the size of a hazel-nut is sufficient for the purpose when introduced into a 4-litre bottle of medium. The muscle may be replaced by a few c.c. of a freshly prepared filtered muscle extract. The evidence as to the exact nature of the substance in muscle which is responsible for this beneficial effect is as yet incomplete.

(*b*) *The Organism.*—There are considerable differences among the strains of *B. welchii* in regard to their toxin-producing capacity. Only a relatively small number appear to be good toxin producers and it is probable that further research is necessary to find a consistently good and reliable strain giving high-value toxin. Bull's strain, 617D, has been found to give very good results in the hands of other workers. There are, however, several strains available at the present time that are quite as good. The capacity of any given strain for producing toxin may be enhanced by increasing its virulence. This can be done by repeated animal passage, preferably through pigeons which are more convenient animals for this purpose than guinea-pigs. The inoculation of a lethal dose of culture into the pectoral muscles of a pigeon produces an illness which is fatal in 6 hours or less. The pectoral region in pigeons is readily accessible and portions of infected muscle can be easily removed under aseptic precautions. An emulsion of infected muscle can be introduced straightway into a pigeon without any intervening culture and thus one may carry out several rapid passages in a short space of time.

It is to be noted that the virulence of a strain may increase by passage up to a certain point and then suddenly fall. This rhythmic

rise and fall in virulence has been noted several times. An organism of ebbing virulence may be set aside in a culture tube in the hope that when next it is used for passage the tide may have turned. A feature which all strains of *B. welchii* seem to possess at some period or other of their growth in artificial media, is the tendency to produce a sticky gelatinous material, the exact nature of which is as yet uncertain. The phenomenon as it occurs in liquid cultures of *B. welchii*, and the peculiar characters of the surface colonies of an organism which is thus affected have been already noted. The tendency to produce this sticky material in no way interferes with the toxin producing capacity of an organism, but it does place a very formidable barrier in the way of successful filtration. On the assumption that an organism in this state might be 'sugar-sick', attempts have been made to rid it of this ailment by long cultivation in carbohydrate-free media, but without any appreciable result. Miss Lacey has found, however, that if a sticky organism be washed repeatedly and kept in normal saline solution for a week in the ice chest, it loses the habit of forming glutinous cultures.

(*c*) *The Inoculum.*—The inoculation of a medium may be conveniently carried out by introducing a piece of infected pigeon muscle taken from a bird that has died as the result of infection. This method has been adopted during the war by the staff at Brockwell Hall in the preparation of toxin in large quantities, and has been found to yield consistently good results. The titre of the toxin may be still further improved by introducing a large dose of the weak broth culture (100 c.c. to a 4-litre bottle) obtained from the preceding pigeon passage.

(*d*) *The Conditions of Growth.*—It is not necessary to adopt special anaerobic methods in growing *B. welchii* for the purpose of producing toxin. The meat medium already mentioned, if freshly autoclaved or re-steamed just before use, offers the conditions suitable for growth. The inoculum for each 4-litre bottle consists of a small piece of infected pigeon muscle plus 100 c.c. of culture. The cultures are allowed to incubate at 37°–38° C. for 16 to 24 hours and then filtered. Cultures yield just as good toxin formation if grown at 42° C.

(*e*) *Filtration.* Cultures are passed through layers of paper pulp in large porcelain cylinders until the filtrates are quite clear and translucent. The material is then passed through Berkefeld filters, after which it is tested for sterility. It should be stored in a cool place away from the light.

B. *Vibrion septique toxin*
B. oedematiens toxin

The conditions which seem to determine the formation of these toxins are identical with those which obtain for *B. welchii* toxin. The same meat broth medium may be employed, the inoculum consisting as before of infected muscle plus culture.

Miss Robertson has been able to prepare very high titre *vibrion septique* toxin using a plain broth or glucose broth medium which has been adjusted to a reaction of P_H 7·8 to 8·0, and which has been inoculated with bits of infected liver taken from guinea-pigs dead of *vibrion septique* infection.

Lastly, it cannot be too strongly emphasized that the factors which govern toxin formation *in vitro* are still beyond our control. However careful and exact one's preparations may be, the result appears still to be a matter of hazard.

2. The Properties of the Toxins elaborated by B. welchii, Vibrion septique, and B. oedematiens.

(i) *Physical Features.*

1. These toxins are all thermolabile and may be destroyed by heating to 70° C. for 30 to 60 minutes.

2. *B. welchii* toxin is reported by Bull as being non-dialysable. No similar evidence is as yet available in regard to the toxins of *vibrion septique* and *B. oedematiens.*

Each of the toxins mentioned depreciates considerably in potency if passed repeatedly through Berkefeld candles and it may be concluded therefore that each is likely to be non-dialysable.

3. All the three toxins are very susceptible to low concentration of acid. *B. oedematiens* would appear to be the most sensitive and *vibrion septique* toxin the least vulnerable, while the toxin of *B. welchii* would seem to occupy an intermediate position in this respect.

In each case the toxin appears not to be precipitated out of solution by contact with acid but to be actually destroyed. There is no clear evidence that it is converted into toxoids.

B. welchii toxin is said to be readily destroyed by free chlorine.

(ii) *Biological Characteristics.*

A. *The toxin of* B. welchii.

1. It is *haemolytic* both *in vitro* and *in vivo*. The *in vivo* action is best seen in animals which have had an intravenous inoculation, though it can also be demonstrated constantly in mice which die as the result of an intramuscular dose of toxin.

Bull relates the case of a rabbit which received a dose of 1 c.c. intravenously at 10 a.m. and which had then a red corpuscle count of 5·4 millions. A progressive destruction of red cells occurred, until, 7 hours after inoculation, the count was only 1·0 million per c.mm. Also, a pigeon (quoted by Bull) which gave an initial count of 4·28 millions red corpuscles was found 3½ hours after inoculation to have no more than 0·8 million red cells per c.mm. in its blood.

The haemolytic action of the toxin is not due to the presence of acids, for it can be demonstrated equally well with toxins that have been carefully neutralized.

Haematuria is a constant finding in mice; it is less frequent in rabbits and in guinea-pigs.

2. It is *necrotic*. This action is best seen in the case of muscle, though it can be demonstrated also in skin by intracutaneous inoculation. The muscle becomes oedematous, friable, and necrosed so as ultimately to form a diffluent pulp.

3. It produces a marked serous oedema which may be found in large quantities in the subcutaneous tissues. This oedema fluid

when collected is found to be almost free from cells. It clots readily and yields a clear serum which is often of a bright yellow colour. In mice, as a rule, the fluid is blood-stained and gives after centrifuging the spectrum of oxyhaemoglobin.

4. It stimulates involuntary muscles. A rabbit which has received an intravenous dose of toxin exhibits violent intestinal peristalsis accompanied by diarrhoea. A similar reaction, though less common, occurs in mice and in guinea-pigs. The vomiting which occurs in pigeons and which may be such a marked feature of the terminal stages of gas gangrene in man is probably explicable on the same grounds.

5. Washed surface growths of *B. welchii* do not produce infection in laboratory animals even when inoculated in large doses. It has been found also that the washed bacilli separated out of a liquid culture are, by themselves, non-infective. If, however, an emulsion of washed bacilli be mixed with a sub-lethal dose of toxin, then there can be induced a typical gas gangrene infection which is rapidly fatal. The toxin therefore stimulates the development of *B. welchii* in the animal body and in this respect has aggressive properties.

Only a very small number of bacilli under these circumstances is requisite for initiating infection. On the other hand the amount of toxin that is necessary for the combination to be successful is a considerable fraction of the lethal dose.

It is probable that there are two contributory factors involved in the mechanism of the aggressive reaction, viz. (*a*) the necrosis of tissue which need not necessarily be looked upon as specific (Bullock and Cramer (1)); (*b*) the antiphagocytic property of the toxin which is specific. Both these effects can be completely inhibited by antitoxin.

6. The toxin has *antigenic* properties.

(*a*) By repeated injection of toxins into animals it is possible to set up an active immunity.

(*b*) Under the same circumstances an animal produces an antiserum which can be shown to possess the following properties.

I. It neutralizes toxin *in vitro*, so that a mixture of toxin with the proper proportion of antiserum is innocuous when inoculated into animals.

Such an antiserum is capable of neutralizing any *B. welchii* toxin.

II. The antiserum when inoculated into animals produces a passive immunity to *B. welchii* toxin and also to *B. welchii* infections. Protection against the lethal effect of toxin was found by Bull to persist for two weeks after the giving of serum.

The prophylactic use of the serum against actual infection was clearly demonstrated by Bull in the following experiment. Guinea-pigs which had received a small dose of serum were found on the first day after inoculation to be capable of resisting at least 150 minimal lethal doses of culture. On the fifth day they survived 300 lethal doses, on the eighth day 60 doses, and on the eleventh day 20 doses.

III. The serum can be used therapeutically to treat the disease when this is already established. Experiments by Bull and others have proved that, if large guinea-pigs be used, a small dose of serum given 24 hours after the inoculation of a lethal dose of culture ensures complete recovery.

B. *The toxin of* vibrion septique.

This toxin is haemolytic, myolytic, and oedema-producing, but to a less degree than *B. welchii* toxin. On the other hand, it does not kill laboratory animals when given subcutaneously or intramuscularly except when inoculated in very large doses. Its lethal effect is best demonstrated by intravenous injection.

Its antigenic properties are in general the same as those outlined for *B. welchii*, with this important difference. An antitoxic serum to *vibrion septique* does not appear to protect if it is given to an animal after the infection is established.

C. *The toxin of* B. oedematiens.

The distinctive feature of this toxin is the extensive and heavy gelatinous oedema it produces both in muscle and in subcutaneous tissues. It kills in small doses and gives rise in horses to an antiserum which is relatively more potent than those so far obtainable against *B. welchii* toxin or *vibrion septique* toxin. In this respect it more closely resembles the toxin produced by *B. tetani*.

3. The Symptoms and Pathological Changes produced by these Toxins and the Estimation of their Minimal Lethal Dose in Animals.

(i) *The Toxin of* B. welchii.

A. *Mice.*

Intramuscular inoculation of a multiple (5 to 10) of the lethal dose is followed by urgent dyspnoea and death with convulsions in 2 to 3 hours. In these animals at post mortem, the local lesion may be very slight or absent, and the appearance of the viscera is normal. With a smaller multiple of the lethal dose (2 to 5) death occurs in 6 to 8 hours. With one lethal dose there is produced an illness which lasts for about 48 hours.

The progressive oedema which develops at the site of inoculation within a few hours and which extends rapidly upwards into the subcutaneous tissues of the abdominal wall is easily palpable. The inoculated limb is held stiffly with the toes firmly flexed. In 24 to 48 hours the animal refuses food, loses weight, and has a staring coat. There is a moderate degree of diarrhoea, and haematuria is frequent. The limb and trunk muscles become paretic so that the animal totters in attempting to walk. Dyspnoeic periods with violent and laboured respiratory movements are an almost constant feature of the illness in its later phases. Ultimately the animal succumbs with a flaccid paralysis of all its limb muscles.

The post-mortem findings in such an animal are as follows: A blood-stained oedema fluid is found extending from the site of inoculation into the opposite groin and upwards into both axillae. The muscles of the inoculated limb are pale, friable, and necrosed, but are never reduced to the liquid grumous state that is such a marked feature in cases of actual infection. The bladder is usually distended with urine which is deeply stained with blood. The kidneys

are reddened and engorged and bulge from their capsules when cut into. The liver may show fatty degenerative changes either in patches or diffusely distributed throughout the whole viscus. There may be an increased amount of fluid in the serous cavities, more particularly in the pleura and in the pericardium. The duodenum and part of the small intestine are distended with liquid contents which are heavily bile-stained. The rectum is often filled with gas and semi-fluid contents. The suprarenals may be reddened and the spleen may be much enlarged but both these changes are inconstant.

The titration of *B. welchii* toxin as carried out by the intramuscular inoculation of mice is exemplified in the following tabulated experiment which was undertaken to estimate the potency of the toxins produced by five different strains of the organism.

Strains.	*Amounts of toxin c.c.*							*Minimal lethal dose.*
	1·0	0·8	0·6	0·5	0·4	0·3	0·2	
B. 85 . . .	++			++	−−	+−	−−	0·5 c.c.
B. Petric . . .	++	−−	+−	−−				1·0 c.c.
B. King . . .	++	++	++	+−				0·6 c.c.
B. Gorman . .	++	+−	−−	−−				1·0 c.c.
B. Quirk . . .	++			++	++	++	−−	0·3 c.c.

+ =death within 48 hours. − =recovery.

Two animals were used in every case.

Subcutaneous inoculation of *B. welchii* toxin into mice produces an illness which is exactly similar to that which follows on intramuscular injection.

The intravenous inoculation of mice with lethal doses of the same toxin would seem to give irregular results. Death may occur in a few minutes or after many hours, so that it becomes by no means easy to determine the exact minimal lethal dose.

B. *Rabbits.*

One minimal lethal dose of toxin given intravenously to a full-grown rabbit produces death in 2 to 2½ hours. A small percentage of rabbits may survive for a longer period, but as a rule, if death does not occur within 3 hours, the animal recovers completely. With multiples of a minimal lethal dose death may follow in ½ hour. In this case the symptoms are those of muscular excitation and restlessness, with marked dyspnoea and convulsive seizures ending in death.

The inoculation of one minimal lethal dose is followed immediately by a period of dyspnoea ; but this is usually transitory and passes off in a few minutes. Usually no symptoms manifest themselves for 1 to 1½ hours. The animal then becomes restless and changes his position frequently. It is attacked with recurring periods of breathlessness, which, though at first slight, soon become longer in duration and severer in degree. At the same time it develops muscular tremors and paresis. It lies down, slightly to one side, with all its limbs fully extended. The intestines can be seen in violent peristaltic movement under the flaccid muscles of the belly wall. It defaecates frequently and may have actual diarrhoea with haematuria. The head is thrown back into the nape of the neck in a position of opisthotonos, and the nose is raised high so as to

relieve air-hunger. Towards the end, the flaccidity of the muscles becomes absolute. It can no longer raise its head, nor can it recover its position when this is altered by the observer. The respiratory embarrassment becomes accentuated. Periods of shallow rapid breathing give way to laboured efforts, separated by longer and longer phases of apnoea. Finally there occur a few convulsive spasms and the animal is dead.

With the exception of a small amount of blood-stained fluid in the peritoneal cavity, there is very little to be found that is characteristic when these animals are examined post mortem. There may be collections of fluid in the pleural and pericardial sacs. The urine is at times blood-stained, and exceptionally the serum may be tinted with haemoglobin. The rectum may have liquid and gaseous contents. Otherwise no naked-eye changes are to be observed.

C. *Guinea-pigs.*

The illness which results in guinea-pigs after the intramuscular inoculation of *B. welchii* toxin resembles in its main features that already described in mice. The post-mortem appearances are much the same, except that the effects of haemolysis are often less obvious, while reddening and enlargement of the suprarenals are probably more common. Numerous instances of the titration of *B. welchii* toxin by this method can be found in the experimental data recorded by Bull and Pritchett and by De Kruif and his colleagues.

The intravenous inoculation of guinea-pigs does not appear to afford a reliable means of titration, because it is found difficult to fix a time limit for the recording of a lethal effect.

D. *Pigeons.*

Pigeons succumb to a lethal dose of toxin, given intramuscularly, in from 4 to 8 hours. A multiple of the lethal dose may produce death in 3 hours. When introduced by the intravenous route the toxin kills more rapidly (Bull and Pritchett).

The titration of a stable *B. welchii* toxin, precipitated out of solution by absolute alcohol, is given in the subjoined table, the numbers given representing milligrammes of the dried product.

Mice intramuscular.	*Guinea-pigs intramuscular.*	*Rabbits intravenous.*	*Pigeons intramuscular.*
3·0++	10+	50+	5·0+
2·0++	5++	30+	2·5++
1·0++	3+—	20++	2·0+—
0·8——	2·5——	15—	1·5——
0·5——	1·0——	10—	1·0—
0·5——			
m.l.d. =1·0	m.l.d. =5·0	m.l.d. =20	m.l.d. =2·5

(ii) *The Toxin of* Vibrion septique.

It would seem to be so difficult to prepare a *vibrion septique* toxin which when inoculated subcutaneously or intramuscularly will kill the animals that are commonly used for experiment, that most observers have had recourse to intravenous injections. Where mice and guinea-pigs are inoculated by this route, the problem of setting a satisfactory time limit in estimating the significance of a positive

result presents just as great a difficulty as that which occurs with *B. welchii* toxin under similar circumstances. On the other hand where rabbits are used, there is less uncertainty in this matter because these animals either die in a few minutes or recover completely.

The illness in rabbits has many features in common with that which follows the injection of *B. welchii* toxin. There are, however, certain characteristic points which distinguish it from the latter.

1. The incubation period varies from a few seconds up to 20 or 30 minutes.

2. The clinical course of the illness is more fulminant in its manifestations. The disease tends to be convulsive rather than paralytic in character. Within a few seconds or minutes of receiving an intravenous injection, a rabbit may suddenly leap from the cage, perform a series of violent convulsive movements about the floor and drop dead in a corner of the room. Or again, the course of the illness may exactly resemble that produced by *B. welchii* toxin, except that it is compressed into a few minutes instead of being spaced over an hour or more.

These animals show no special pathological changes post mortem. Exceptionally, where death has been delayed for several hours, small amounts of blood-stained fluid may be seen in the serous cavities.

In view of the necessity for testing large numbers of toxins during the war and the difficulty of obtaining rabbits in sufficient numbers for this purpose, there has been elaborated at the Wellcome Physiological Research Laboratories a method of titrating *vibrion septique* toxin which depends on the estimation of the minimal oedema causing dose in mice. It has been found possible to determine this amount of toxin with sufficient accuracy to give reliable comparative values in the standardization of different antisera. The unit of *vibrion septique* antitoxin has been designated as that amount of serum which, when mixed for one hour at room temperature with 10 minimal oedema-causing doses of toxin, will completely inhibit the production of oedema in the animal which is inoculated with the mixture. The method, however, is one that necessitates constant practice, and the results are open to wide differences in interpretation which depend entirely on the experience of individual observers.

(iii) *The toxin of* B. oedematiens.

This toxin kills laboratory animals when given intravenously, subcutaneously, or intramuscularly. It can be very easily titrated by the intramuscular injection of mice, the final readings in regard to a positive or negative result being recorded at the end of 48 hours as in the case of *B. welchii* toxin.

Animals which succumb to *B. oedematiens* toxin show the same massive gelatinous oedema that characterizes a fatal infection by this organism. The muscles at the site of inoculation are much swollen with oedema fluid. They are pale, and often occupied by minute punctiform haemorrhages, but are not necrosed. Small effusions into the serous sacs are common.

The subjoined table represents the titration of a *B. oedematiens* toxin carried out in mice.

Toxin LE 235

c.c.	
0·01	+ +
0·005	+ +
0·002	+ +
0·001	+ +
0·0005	+ +
0·0002	+ +
0·0001	— —

4. The Standardization of Antisera.

(i) B. welchii *antitoxic sera.*

Bull estimated the titre of his antitoxic sera by determining the smallest amount of serum that was requisite to neutralize one minimal lethal dose of toxin in a 250 grm. guinea-pig or a 300 grm. pigeon. This amount of serum he has defined as the unit of antitoxin.

Where large numbers of different sera have to be tested this method becomes a very expensive procedure. To obviate the difficulty, the Wellcome Physiological Laboratories have used mice in place of guinea-pigs and pigeons. As has been already indicated the minimal lethal dose of *B. welchii* toxin can be readily estimated in mice. For testing purposes at the W.P.R.L. the unit of antitoxin has been fixed as being twice the amount of serum that is sufficient to neutralize the lethal effect of two minimal lethal doses, the mixture of toxin and serum being left in contact for one hour at room temperature before inoculation. The results obtained by this method are claimed to yield a figure in unitage which within reasonable limits is identical with that obtained when the same sera are tested according to Bull's method.

A. Titration of *B. welchii* toxin LW. 218.

c.c.	
0·4	+ +
0·3	+ +
0·25	+ + + +
0·2	+ — — —
0·15	— —
0·1	— —
0·1	— —

m.l.d. = 0·25c.c.

B. Titration of antisera against two minimal lethal doses, viz. 0·5 c.c.

	SERA			
	115B	25V	14b	810
0·1	+ +	+ +	— —	— —
0·01	+ +	+ +	— —	— —
0·005	..	..	— —	..
0·004	..	..	— —	..
0·003	..	..	+ —	..
0·002	..	..	+ —	..
0·001	+ +	+ +	+ +	— —
0·0008	..	..	..	— —
0·0006	..	..	..	— —
0·0005	..	..	..	— — — +
0·0004	..	..	..	+ + + +
0·0002	..	..	..	+ +
0·0001	..	..	..	+ +
Unitage	0	0	125	1,000

+ = death in 48 hours. — = recovery.

The previous record shows an evaluation of some sera thus tested. The sera designated 115B, 25V, and 14b were German anti-gas gangrene sera captured on the Western Front, while 810 is a single horse serum prepared by Weinberg. The latter in addition to *B. welchii* antitoxin contains antitoxin to *vibrion septique* and to *B. oedematiens.*

(ii) Vibrion septique *antitoxic sera.*

French workers have defined as the unit of *vibrion septique* antitoxin the smallest amount of serum which will neutralize the fatal effect of one lethal dose of toxin given intravenously to a full-grown rabbit, the mixture of toxin and serum being kept at room temperature for one hour before inoculation.

The following record illustrates the titration of Weinberg's serum 810 by this method.

A. Titration of toxin.

2·0 c.c.	+ 7 min.
1·5 c.c.	+ 15 min.
1·5 c.c.	+ overnight
1·0 c.c.	recovery
1·0 c.c.	recovery

B. Titration of serum against 5·0 c.c., i. e. 3 certain minimal lethal doses.

serum.	*result.*
0·01	recovery
0·005	recovery
0·004	recovery
0·004	recovery
0·003	+ 20 mins.
0·003	+ overnight
0·002	+ 8 min.
0·001	+ 3 min.

The serum then is found to neutralize in such a way that 0·004 gives complete protection against 3 m. l. d., i. e. it contains approximately 750 units.

(iii) B. oedematiens *antitoxic sera.*

	25V	115B	124	410	411	810
0·1	+ +	— —	— —	— —	— —	— —
0·01	+ +	+ +	+ +	— —	— —	— —
0·005	+ +	+ +	+ +	+ +	— —	— —
0·002	+ +	+ +	+ +	+ +	— —	— —
0·001	+ +	+ +	+ +	+ +	+ —	— —
0·0008	..	..	..	..	+ +	— —
0·0006	..	..	..	..	+ +	— —
0·0004	..	..	..	..	+ +	— —
0·0002	..	..	..	..	+ +	— —
0·0001	..	..	..	..	+ +	— —
0·00008	..	..	..	..	+ +	— —
0·00006	..	..	..	..	..	— —
0·00004	..	..	..	..	..	— —
0·00002	..	..	..	..	..	+ +
0·00001	..	..	..	..	..	+ +
	0	5	5	50	250	8,000

+ =death in 8 hours. — =recovery.

Weinberg has defined as the unit of antitoxin the smallest amount of serum which will completely neutralize 100 minimal lethal doses of toxin in a mouse, the mixture being kept for one hour at room temperature before inoculation. The accompanying record illustrates an experiment in which various sera were titrated against a test dose consisting of 50 minimal lethal doses of toxin.

5. Preparation and Standardization of Anti-gas gangrene Sera.

(*A Report by the Staff of the Wellcome Physiological Research Laboratories*).

(i) *Introduction.*

The investigation into the possibility of making anti-gas gangrene serum was first undertaken at the request of the Medical Research Committee, and the immunization of horses was commenced in October 1917. Dr. McIntosh on behalf of the Committee, supplied suspensions of surface growths, and also broth cultures of the various anaerobes considered to be concerned in the production of gas gangrene. He added solutions of iodine to these cultures to produce attenuation. Two horses were immunized with these cultures over a period of some months. At one stage, when the horses had been under immunization for several months, and supplies of the bacterial suspension were not available, *B. welchii* toxin was injected. Prior to this time, the *B. welchii* antitoxic titre of sera from these two horses was low. What potency the sera of these horses possessed when issued for field trial in France in March 1918 was, therefore, the result of immunization with cultures and toxin in the case of *B. welchii*, and of cultures only in the case of *vibrion septique*, and other organisms. In January 1918, Major Carrol Bull of the United States Army had demonstrated in London the power of *B. welchii* antitoxin to neutralize the toxin of *B. welchii*. Bull presented a culture and a sample of antitoxin to the Laboratories. *B. welchii* toxin was rapidly prepared in large quantities for injection into horses. Antitoxin of sufficiently high titre to protect animals against large doses of living culture of *B. welchii* was available for field trial in March 1918.

Meanwhile, evidence of the importance and frequency of the occurrence of *vibrion septique* was accumulating. At a War Office conference in March 1918 the pathologists present agreed that this organism was frequently present in wounds and was the cause of severe and fatal cases of gas gangrene occurring in soldiers. Work was immediately begun at the Laboratories with the aim of producing an antiserum to *vibrion septique*. Cultures of *vibrion septique* were presented by Dr. McIntosh, Miss Robertson, and Capt. Henry, and samples of toxin for testing purposes by Miss Robertson. Large quantities of toxin were made at the Laboratories for injection into horses, and by May 1918 a serum had been produced which was of sufficiently high titre to protect an animal, which had received some hours previously 5 c.c. of serum, against many lethal doses of toxin or culture. Some of the horses yielding this serum had already been immunized with *B. welchii*. Thus, a double serum containing

antitoxins to both *B. welchii* and *vibrion septique* became available, and was ready for trial in May 1918.

B. oedematiens toxin made by Captain Henry from cultures isolated by him, became available in quantity in June 1918. Serum of satisfactory protective value, as judged by results obtained with laboratory animals, was produced in September 1918.

The aim throughout the whole of the work was to produce as rapidly as possible a serum which, when injected into a soldier immediately after the infliction of a wound, might protect him against the onset of gas gangrene.

It was decided at an early date to include the recognized prophylactic dose of tetanus antitoxin in the prophylactic gas gangrene serum. The laboratory staff commenced in February 1918 the preparation of T.W. serum, i.e. one containing sufficient antitoxins to both *B. tetani* and *B. welchii* to protect a laboratory animal against many lethal doses of cultures of these two organisms.

T.W. serum was produced in quantity in March 1918. T.V.W. serum, containing antitoxin for *B. tetani, B. welchii,* and *vibrion septique,* was produced in August 1918. T.V.W.E. serum, containing antitoxin also to *B. oedematiens* became available only about the date of the armistice in November 1918.

It may here be remarked that in 1918 almost the whole of the work was directed to the production of antitoxic, as opposed to antibacterial sera. It early became evident that the practical difficulties of preparing, in the very large quantities required, the 'whole cultures' of bacilli to a constant standard of pathogenicity, so that the injections into horses would proceed smoothly and safely, were so great that the production of sera for field trial in large quantities would inevitably have been delayed for many months. It was found that antitoxins of high titre, when injected into animals, gave them complete protection against subsequent injection of many lethal doses of living cultures. It was, therefore, considered advisable to concentrate the main effort on the production of purely antitoxic sera while investigating the methods of preparation and evaluation of antibacterial sera. The termination of the war came before the experimental work with antibacterial sera was very far advanced.

From the foregoing account it is clear that the sera actually tested in the British Army in France and used prophylactically and curatively were the first results of an attempt to produce three new immune sera. The nature of the toxins were only obscurely understood and very serious difficulties both practical and theoretical had to be encountered. The sera were definitely not the final and most potent products possible, it is not even now known what titre should be considered to be a useful minimum. In judging the results therefore of the serumtherapy it must be borne in mind that the tests were made at the earliest possible moment and that rightly considered no really adequate therapeutic trial has been made of these sera.

(ii) *Preparation of Antitoxins.*

A. *B. welchii.*

Toxin.—Ordinary peptone meat broth or tryptic digest broth containing 1 per cent. of glucose, was inoculated with a 24-hour

culture of *B. welchii*. The addition of sterile fresh muscle or of autoclaved meat seemed on the whole to be of advantage. The inoculated broth was kept at 37° for 24–30 hours, and then filtered through kieselguhr candles, carbolized and kept in a cool place. It was not difficult to produce large quantities of toxin of which 0·2 c.c. to 0·3 c.c. would, when injected intramuscularly, kill a 250-grm. guinea-pig or a 20-grm. mouse.

It was not found necessary to use any special methods for securing anaerobiosis: the broth was not covered with paraffin. The inoculum was added to the broth a few hours after it had left the autoclave and had cooled to about 40° C.

Immunization of the Horse.

The processes of immunization correspond almost exactly to those in common use for the preparation of diphtheria antitoxin. *B. welchii* toxin was injected intramuscularly at intervals of a few days, a representative series of doses being 1 c.c., 2 c.c., 4 c.c., 8 c.c. at intervals of 2 or 3 days. Ten weeks later the dose of toxin reached 400 c.c. and the antitoxic value of the horse's serum was 1,000 to 2,000 units. With the average horse a titre of 1,000 units was attained in about 8 weeks; at this point the animal was bled.

Serum containing 5,000 units per c.c. was obtained from several horses, but as the object of the work was to produce the maximum quantity of serum with a titre of 1,000 units in a given time, no opportunity was taken of continuing the immunization without bleeding to ascertain the highest titre attainable by this method.

B. *Vibrion septique.*

Toxin.—The method of making toxin was similar to that described for *B. welchii*. The inoculum was either a portion of a culture or the infected breast muscle of a pigeon injected the day previously with a culture of *vibrion septique*.

The toxin produced in large quantities was sufficiently potent to kill a rabbit within ten minutes when a dose of 0·5 c.c. was injected intravenously.

Immunization of the Horse.

The methods closely resembled those used for the production of antitoxin to *B. welchii*, though the rate of increase of dosage was slower. An average horse would produce in 8 to 12 weeks serum containing 1,000 units of *vibrion septique* antitoxin per c.c.

C. *B. oedematiens.*

Toxin.—By using the method above described in the *B. welchii* section, toxin was produced in large quantities of which 0·0001 c.c., injected subcutaneously, would kill a 20-grm. mouse within 24 hours. The toxin is apparently rather unstable, the toxicity rapidly decreasing. Whether this old weak toxin is a good antigen is not at present known with accuracy.

Immunization of the Horse.

The methods in use are exactly comparable with those in common use for the preparation of antitetanic serum. An average horse in 3 months would yield a serum containing 5,000 units of *B. oedematiens* antitoxin per c.c.

(iii) *Methods of estimation of the value of anti-gas gangrene sera.*

A. *Antitoxin of* B. welchii.

The unit in use was based upon a serum supplied by Bull which bore a label indicting the unitage: Bull used as his unit the quantity of serum which neutralized an amount of toxin equal to one lethal dose for the pigeon. We found that this quantity of serum was twice the amount necessary to neutralize twice the lethal dose for a mouse. The unit arrived at in this way was adopted for the titration of *B. welchii.*

Toxins used for standardization would in a dose of 0·1 c.c. to 0·25 c.c., when injected intramuscularly, kill a mouse within 24 hours.

Serum was produced containing 5,000 units per c.c.

B. *Antitoxin of* Vibrion septique.

The toxin produced by *vibrion septique* differs from that produced either by *B. oedematiens* or *B. welchii* in that it does not cause death when inoculated intramuscularly or subcutaneously into mice. It can, however, be readily titrated by intravenous inoculation into rabbits, a high value toxin producing death in 3–15 minutes with a dose of 0·5 c.c. or less when tested by this method.

A delayed reaction in which death occurs after 3 to 24 hours takes place with lesser doses. This delayed reaction is unsatisfactory from the point of view of a test as it is difficult to adjust the dose, and individual animals show a variable resistance. Since the intravenous test became impracticable when the work was carried out on a large scale it was necessary to substitute some other method of titration.

It was found that 0·05 c.c. of toxin injected intramuscularly into a mouse produces a recognizable oedema. This reaction is used to test the potency of the serum. The test dose of toxin used is ten times the amount, i. e. 0·5 to 1 c.c. according to the strength of the toxin. This test dose is equivalent to an intravenous lethal dose for the rabbit. A small number of antitoxins were titrated by: (1) the mouse-oedema method; (2) intravenous injection into mice; (3) intraperitoneal injection into mice; (4) intravenous injection into rabbits. The results agreed sufficiently closely to justify the temporary use of the ' mouse-oedema method '.

C. *Antitoxin of* B. oedematiens.

The unit of antitoxin (which was already in use by Dr. Weinberg at the Pasteur Institute) is that quantity of serum which when added to 100 minimal lethal doses of toxin injected intramuscularly into a mouse will render the toxin inert and prevent the death of the animal. Sera were produced containing 20,000 units per c.c.

We should like to take this opportunity to express our thanks to Dr. Weinberg for his courtesy in sending us samples of his sera on several occasions.

6. The Serum Therapy of Gas Gangrene.

(i) *The Collection of data.*

The treatment of cases of gas gangrene by means of antisera was first carried out in British military hospitals in France during the spring months of 1918. Reports on the results obtained by the use of these antisera were compiled by a number of R.A.M.C. officers and forwarded to the Medical Research Committee. These reports form the basis of the following summary.

The accompanying table (Table I) indicates the number of cases recorded by each observer, together with the number of deaths occurring in each series.

Table I.

Reported by	*No. of cases.*	*No. of deaths.*
Barling	4	4
Brenan	1	1
Ellis	9	3
Hope	1	1
McEwen	1	1
McNee	31	12
Stokes	23	16
Tytler	17	11
Wyard	2	1
Totals	89	50

(ii) *The Serum employed.*

The sera which were used in France were prepared by the staff of the Wellcome Physiological Research Laboratories at Herne Hill. The titre of each serum, i.e., the number of units of *B. welchii* antitoxin and of *vibrion septique* antitoxin contained in each c.c. of serum is set out in Table II.

Table II.

Serum.	B. welchii *antitoxin units.*	V. septique *antitoxin units.*
G 2	2,000	—
G 3	1,000	300
G 17	1,000	300
G 18	500	300
G 19	500	300
G 22	2,000	—
G 44	2,500	1,500
G 53	1,000	3,000

It is to be noted that in the titration of the above sera the unit of *B. welchii* antitoxin is taken as being twice the amount of serum requisite to neutralize two minimal lethal doses of toxin in the mouse. This method of measurement has been found to yield a result which within reasonable limits is identical with the figure obtained by Bull's method of titration.

The unit of *vibrion septique* antitoxin is estimated as being the amount of serum which will completely neutralize 10 minimal-oedema-causing doses of toxin in the mouse. This procedure yields a much higher figure than that obtained by the French method of standardization, in which the antitoxin unit is taken as the amount of serum which will neutralize one intravenous minimal lethal dose of toxin in a full-grown rabbit.

The sera designated as G3, G17, G18, and G19 were prepared by the inoculation into horses of mixed cultures of anaerobes which had been attenuated with iodine. These attenuated mixed cultures were furnished by McIntosh. The resulting sera proved to have a low antitoxic value. The amount of *vibrion septique* antitoxin did not pass beyond the 300 unit level, while the titre of *B. welchii* antitoxin remained in the neighbourhood of 100 units per c.c. It was only after treating these same horses with *B. welchii* toxin that the content in antitoxin as set out in Table II was reached. The sera referred to are claimed by McIntosh to have distinct advantages over purely antitoxic sera in that they contain antibacterial bodies.

The production of sera by the inoculation of attenuated whole cultures of anaerobes was found to present considerable difficulties when carried out on a large scale, and the method was abandoned in favour of inoculation with sterile toxins. The sera designated G2, G22, G44, and G53, thus prepared, were therefore purely antitoxic sera with no antibacterial content.

None of the sera issued for use in the field contained antitoxin to *B. oedematiens*.

The sera designated *B.* in section (vi) were *B. welchii* antitoxic sera prepared by Bull in the Rockefeller Institute and had a titre of *B. welchii* antitoxin varying from 750 to 1,800 units per c.c.

(iii) *Analysis of Records.*

The serum therapy of cases of gas gangrene became available at a period in the history of the war when it had already been clearly demonstrated that the disease was amenable to rigorous surgical procedures. It so happened then that antisera came to occupy two separate rôles in the treatment of gas gangrene.

1. Where the focus of the disease could be extirpated or otherwise effectively dealt with by the surgeon the use of serum became an adjuvant to surgical measures. If, for example, symptoms of toxaemia persisted after complete removal of all anaerobe infected tissue, recovery could be hastened by the giving of serum. Or again, the giving of serum to a man suffering from serious toxaemia might improve his physical condition sufficiently to permit of operation. In either instance, the beneficial effect of the serum was due to the neutralization of toxin which had already reached the circulation. The serum was not called upon to combat the infection.

2. On the other hand, the serum came to be used in desperate cases, in which, for various reasons, surgical intervention was inadequate to cope with the disease. The task assigned to the serum in this particular class of case was a very severe one, for it had to deal not only with toxin in the circulation and in the infected tissues, but it had also to arrest the actual spread of infection and finally to overcome it.

The whole series of cases, the salient features of which have been set forth in section (vi), can be best analysed by considering, firstly, the cases in which death occurred ; and secondly, the cases which recovered and in which the serum was judged to have been beneficial.

A. *Deaths.*

These may be classified as follows :

1. Cases in which the wounded man recovered from gas gangrene and in which death may be reasonably attributed to some other conditions, such as a streptococcal septicaemia or a pneumonia.

The following cases in section (vi) come under this category :

Case number.	*Day of death.*	*Cause of death.*
38	10	Lobar pneumonia.
45	10	Lobar pneumonia.
73	8	Broncho-pneumonia.
63	9	Streptococcal septicaemia.
74	6	Streptococcal septicaemia.
88	21	Streptococcal septicaemia.
56	—	Streptococcal meningitis.

All these cases had undoubted gas gangrene at the outset, and in each the disease was arrested by a combination of surgery and serum. Although it is impossible to ignore the influence of gas gangrene in establishing such secondary infections in a wounded man, yet it is obvious that these cases should not be looked on as failures in serum therapy.

2. Cases in which a fatal issue is determined by the presence of an anaerobe other than those anaerobes against which the serum has been prepared.

The serum therapy of gas gangrene was started at a time when there was still lacking much information as to the nature of the anaerobes that caused the disease in wounded men. It was known that *B. welchii* could be isolated from the majority of human cases. It was known too that *vibrion septique* could be found in a certain number of cases, but no figures from British sources were available as to the percentage incidence of this anaerobe in wounds. Further, *B. oedematiens*, which Weinberg claimed to have demonstrated in about one-third of his cases, had not been recorded by British workers, with the solitary exception of the case reported by Miss Dalyell.

It became therefore a matter of prime importance, not only to determine in what percentage of cases of gas gangrene a *welchii-septique* antitoxic serum could be expected to give beneficial results, but also to investigate the cause of death in all instances in which liberal and fair trial of the serum proved unsuccessful. The first part of the investigation was completed, and provided a census of the anaerobes causing gas gangrene in man. It showed that in about 25 per cent. of all cases of gas gangrene the disease was due to anaerobes other than *B. welchii* and *vibrion septique*, i. e. in one-quarter of the cases a *welchii-septique* serum could not be expected to combat the infection. The second part of the investigation, which presumably would have yielded evidence complementary to the first part, broke down owing to the difficulty of procuring from France pieces of infected muscle from unsuccessfully treated cases.

Of the 87 serum treated cases only 5 were proved to contain pathogenic anaerobes, infection by which in guinea-pigs could not be arrested by a *welchii-septique* serum ; and there can be no doubt, on the evidence available, that this number would have been considerably augmented if a thorough investigation had been possible.

Cases 48, 54, and 89 contained pathogenic strains of *B. fallax.* Case 84 contained *B. oedematiens,* and an unidentified pathogenic anaerobe was isolated from Case 12.

The details of Case 54 as reported by Stokes are as follows :

Sa. Multiple wounds of thigh. Leg discoloured, crepitations round ankle. Oedema up to groin. Pulse very bad.

28. 4. 18. Operation. Multiple incisions. Calf muscle good. Thigh muscles heavily infected. Given serum 30 c.c. G 17 and 30 c.c. G 22.

29. 4. 18. Vomiting stopped. Feels better. Pulse better. Infection has not spread but crepitations obvious round knee and thigh. Later the foot became black and gangrenous. Given 60 c.c. of serum. He again felt better after the serum. In the evening again given 60 c.c. of serum. Amputation through upper third of thigh. Stood the operation well. He could not have done so 24 hours previously.

30. 4. 18. In the morning fairly well but pulse very weak. Given 40 c.c. G 17. In the evening became delirious and died about midnight. There was no recurrence of gangrene in the stump.

Blood cultures.

28. 5. 18. *B. fallax.*
29. 5. 18. *B. fallax.*
29. 5. 18. (evening) *B. fallax.*
30. 5. 18. (after amputation) negative.

The notes of Case No. 12 reported by Ellis are as follows :

18. 10. 18. Wounded in right leg and right arm.

19. 10. 18. Admitted to hospital. Through-and-through wound of anterior and external part of upper thigh. Also through-and-through wound of right knee-joint. Whole thigh tense, oedematous and tympanitic anteriorly up to Poupart's ligament. No crepitation. Offensive, effervescent fluid bubbles from wound in knee-joint.

Operation. Very incomplete excision of wound. Muscles discoloured but contractile, bleeding muscle reached in some places. Knee-joint flushed with saline and ether and closed. General condition fairly good, colour good, no vomiting, pulse small volume but not exceedingly rapid. Under anaesthetic pulse rapidly ran up to 100.

Serum G 53, 100 c.c. in 1,000 c.c. salt solution intravenously.

20. 10. 18, 3.30 p.m. Has been perfectly clear mentally until the last few hours. He is now slightly delirious. No vomiting, colour bad, sweating, intense thirst, pulse bad. No spread of gas to abdominal wall, but the thigh is more extensively involved and malodorous.

Serum G 53, 100 c.c. in 1,000 c.c. saline intravenously. 7.0 p.m. death.

Autopsy. 3 hours after death.

Anterior muscles of thigh all putrid, posterior muscles full of gas. Gluteal and abdominal muscles not involved.

Bacteriological examination.

Muscle-direct smear, very numerous Gram-positive thick bacilli many showing spores. Spores oval, mostly subterminal but many central.

Meat culture—organism described and *B. sporogenes.*

Blood culture, 9 hours before death, gave the organism described as occurring in direct smear from muscle.

Remarks.

Very severe case of gas gangrene, not benefited by serum. Sporing anaerobe in blood culture.

In a letter accompanying cultures which were sent to the Medical Research Committee, Ellis says :

'This is my first real failure with the serum. I should have laid down a serum barrage in the thigh and should have repeated serum at 9.30 instead of 3.30. However, personally I do not think that in this case the serum was active.'

3. Cases in which the serum was given either in too small amounts or at too late a period in the course of the disease.

Experience with the antisera of which we have most reliable information, viz., those against tetanus and diphtheria, has shown conclusively that they must be given in large doses and at the earliest possible stage in the development of the illness. In the case of diphtheria, Behring and many other workers have demonstrated that with efficient serum treatment in the first two days of the disease the death-rate is reduced to about 7 per cent. The use of serum after two days gives less favourable results, and after the fifth day it is found to exercise little or no beneficial effect. The good fortune which attends the early serum therapy of diphtheria depends to a very large extent on the ease with which the condition can be diagnosed clinically, for the sore throat, which is such a well-marked clinical feature, attracts attention to the probable existence of the disease. Less happy results have been obtained in the treatment of tetanus, because here the disease may develop when it is least suspected, and a clinical diagnosis becomes possible only when grave symptoms are already manifest. It is for this reason that antitetanic serum best demonstrates its efficacy when it is given prophylactically. Now, both in diphtheria and in tetanus the march of events is measured in periods of days. In gas gangrene, on the other hand, the disease may rush relentlessly to a fatal issue in the course of a few hours, so that the wounded man may be practically moribund by the time that serum treatment becomes available.

In searching through the records collected in France one finds a large number of instances in which serum was first administered when the wounded man was really dying. It can be legitimately claimed that under these conditions the serum had no chance of effectively combating the disease.

These moribund cases may be divided into two groups.

Group A.

Acutely fulminating types of the disease where death occurred within three days. The following may be taken as typical samples :

No. of case.	*Day of death.*	*Duration of life in hours after giving of serum.*
17	1	6
31	1	9
? 39	1	10
11	2	4
43	2	11
55	2	7
57	2	5
58	2	2
64	2	6
65	2	8
10	3	6
49	3	6

Group B.

Late cases of gas gangrene in which death occurred after the third day.

No. of case.	*Day of death.*	*Duration of life in hours after giving of serum.*
68	5	4
70	6	6
69	8	½
4	11	6

Group A were treated in forward medical units and represent a type of case which is unavoidable under modern conditions of warfare. Group B were base hospital cases and might conceivably have benefited if serum had been given earlier.

B. Recoveries.

Of these there are 38 cases out of a total of 89 serum treated patients. They may be divided into three groups :

1. Cases in which surgical treatment consisted in thorough cleansing of the wound, together with removal of foreign bodies, excision of infected muscle, &c.

16 cases.

2. Cases in which it was necessary to amputate a limb.

17 cases.

In groups 1 and 2 there are many instances in which beneficial effects are ascribed to antisera, but in the absence of large statistics it is impossible to decide whether surgery plus serum accomplished more than surgery along could have done.

3. Cases in which operative procedures were insufficient to arrest the disease, or in which the focus of infection was inaccessible.

5 cases.

Of these 4 were reported by Ellis and 1 by Tytler. It is to be noted that both workers gave large and repeated doses of serum, that the serum Ellis used for his series was the best of the sera sent out to France, and that he used it locally in and around the infected tissues.

One of the cases treated by Ellis and Tytler's case are reported in full.

Case No. 13. St.

Multiple shell wounds, both legs and thighs. Double fractured femur. G.S.W. scalp. Wounded morning 17. 10. 18.

19. 10. 18, 3.30. Patient in very bad condition, delirious and wildly excited ; colour definitely yellow. Pulse, bad quality, 140. Left thigh gangrenous with green discolouration below upper third. Left leg greenish black and absolutely cold to the knee. Very marked oedema and crepitation over whole thigh anteriorly extending up to groin with marked tympany on percussion, and marked reddening of the skin and bronzing. Posterior thigh muscles in upper third soft, glutei soft. Patient inoperable. 100 c.c. serum in 1,000 c.c. salt solution intravenously and 30 c.c. intramuscularly in gluteal adductor muscles and in anterior abdominal wall.

10.00. Remarkably improved, now perfectly clear mentally, pulse 112. Gas not spreading, or very slightly, some increase in skin redness especially over glutei. Oedema and crepitation if anything diminished.

Spinal novocaine anaesthesia amputation in middle third of thigh immediately above line of green discolouration. Adductors and vasti discoloured, contraction sluggish, other muscles appear fairly healthy. Collapse following amputation, pulse and respiration ceased, patient practically dead. Blood transfusion 700 c.c. with dramatic improvement.

15.30. Sleeping quietly, pulse fair volume, 116. 60 c.c. serum intramuscularly (40 c.c. in pectorals, 20 c.c. in thigh and buttocks).

19.30. Blood transfusion 800 c.c.

20. 10. 18. General condition good, full strong pulse, rate 112. temperature rising, sweating. Mental condition absolutely clear. The area of skin redness is spreading and now extends some distance up left abdominal wall and up back over lumbar muscles. There is, however, no oedema or tenderness and the thigh feels quite soft though tympanitic. There is a bleb on anterior abdominal wall just above the area of redness. 15.50. 80 c.c. serum intramuscularly (60 c.c. pectorals and 20 c.c. in abdominal wall and back).

23.00. Vomiting since about 15.00, now vomiting almost continuously. Wound dressed, whole stump swollen with superficial crepitation, muscles all red, healthy and contractile except adductors, ends of which show superficial gangrene, beneath this muscle discoloured, soft, oedematous and non-contractile. 50 c.c. serum in 500 c.c. salt solution intravenously.

21. 10. 18, 22.00. Much improved, vomiting eased off through the night and to-day he has not vomited at all. Colour good. Evening temperature 99·6, pulse 90. General condition excellent. The redness over anterior abdominal wall and lumbar muscles has now entirely faded. Wound dressed, healthy except for adductors which are absolutely pulped, spongy, and full of gas. Dead muscle dissected away without anaesthetic. Other leg multiple wounds dressed, fractured femur put up in Thomas. 60 c.c. serum intramuscularly.

Bacteriological Examination.

Muscle 19. 10. 18, 8.30. Direct smear, non-sporing anaerobe, morphologically resembling *B. welchii*. Fairly numerous diplococci culture, anaerobes probably *B. welchii* and *B. sporogenes*.

Muscle, 20. 10. 18, 23.00. Direct smear. Predominant organism a very large, very broad strongly Gram-positive bacillus with rounded ends, some show tendency to oat shape. Spore forms are fairly numerous and are chiefly subterminal but some are central. There is also a long slender bacillus with round terminal spore. Fairly numerous cocci in pairs and short chains.

Remarks.

Desperate case, successfully treated. Large doses required and recurrence of signs of intoxication and local spread of gas when serum was not pushed, immediate improvement with increased dosage. All officers who have seen this case consider the recovery extraordinary and agree that they have never seen anything like it before.

Case No. 76. Ke.

24. 4. 18. G.S.W. Buttock.

27. 4. 18. Admitted. P. 104, T. 103.

28. 4. 18. X-ray shows large F.B. in left back. Vomiting. T. 104, P. 100.

29. 4. 18. Operation. Wound excised and excision entended up over crest of ileum, which was slightly shattered. Large F.B. removed from body of psoas. Flavine gauze drainage. Probable gas infection.

Exploratory incision through left rectus abdominis shows no lesion of bowel or peritoneum but some serous fluid.

Vomited once, 3 p.m. 8.0 p.m. T. 99, P. 120.

Urine shows considerable albumin and a good number of granular casts.

30. 4. 18. Condition fair. Not much sleep. a.m. T. 102, P. 120.

No vomiting. p.m. T. 101, P. 114.

1. 5. 18. Better night, but complains of pain in side, a.m. T. 97, P. 104.

No vomiting. p.m. T. 103, P. 128, R. 32.

Urine shows trace of albumin and occasional granular casts.

2. 5. 18. Condition not so good. Very restless. No vomiting. a.m. T. 99·4, P. 78, R. 30.

Left scrotum and inguinal canal show marked well demarcated swelling and tenderness.

Operation. Inguinal canal opened. Coverings of cord contain pus and gas. Vessels of cord thrombosed throughout. Testicle and cord stripped out of scrotum. Incision extended upwards to abdominal wall and cord followed up retroperitoneally. Large abscess between ileo-psoas fascia and peritoneum extending into pelvis to base of bladder and upward in loin to former site of F.B. in psoas muscle. Deferential vessels are natural from internal inguinal ring to base of bladder. Spermatic vessels thrombosed as far as renal vessels and perivesicular tissue is oedematous. Deferential vessels tied off at brim of pelvis and spermatic vessels at lower pole of kidney. The testicle and cord as removed are completely gangrenous and show definite gas. Cultures from this tissue yield streptococcus and B. welchii.

3. 5. 18. Slept fairly well. Vomited in morning. a.m. T. 98, P. 120, R. 30. p.m. T ?102·6, P. 112, R. 28.

2.30 p.m. 20 c.c. serum (W.G. 22) intravenously. Dressed. Showed some reaction with chill 30 minutes later.

4.30 p.m.	20	c.c.	serum	(W. 22)	intramuscularly.
6.30 ,,	20	,,	,,	,,	,,
7.30 ,,	20	,,	,,	,,	,,
9.0 ,,	20	,,	,,	,,	,,
10.0 ,,	20	,,	,,	,,	,,
11.0 ,,	30	,,	,,	,,	,,
2.0 p.m.	T. 103·4	P. 126	R. 26.		
6.0 p.m.	T. 101·2	P. 90	R. 30.		
10 p.m.	T. 102	P. 128	R. 30.		

4. 5. 18. Poor night. Very restless, irrational. Bowels moving constantly. Vomited after liquid food in morning. Seems distinctly improved generally, however.

12 noon	10	c.c.	serum	(W.G. 22)	intramuscularly.
2.0 p.m.	10	,,	,,	,,	,,
6.0 p.m.	10	,,	,,	,,	,,
9.0 p.m.	10	,,	,,	,,	,,

Slept at intervals during the afternoon. Takes nourishment better. No vomiting.

2.0 a.m.	T. 101	P. 120	R. 30.
6.0 a.m.	T. 102·4	P. 118	R. 28.
10.0 a.m.	T. 102	P. 120	R. 28.
2.0 p.m.	T. 102	P. 120	R. 32.
6.0 p.m.	T. 102·4	P. 120	R. 36.
10.0 p.m.	T. 101·8	P. 118	R. 30.

5. 5. 18. Rested more quietly. Slept at intervals. Took a fair amount of nourishment and has not vomited.

1.0 a.m. 10 c.c. serum (W.G. 22) intramuscularly.
6.0 a.m. 10 ,, ,, ,, ,,
8.0 a.m. 10 ,, ,, ,, ,,
12 noon. 10 ,, ,, ,, ,,
2.0 p.m. 10 ,, ,, ,, ,,
4.0 p.m. 10 ,, ,, ,, ,,
7.0 p.m. 10 ,, ,, ,, ,,
10.30 p.m. 10 ,, ,, ,, ,,
2.0 a.m. T. 104·4 P. 112 R. 32.
6.0 a.m. T. 100 P. 114 R. 30.
12 noon. T. 100·8 P. 106 R. 26.
6.0 p.m. T. 104 P. 130 R. 26.
10 p.m. T. 103·7 P. R.
Vomited after food—4.30 p.m. and 6.30 p.m.

6. 5. 18. Good night. Slept fairly well. Condition about the same. Takes nourishment well. Wound rather dirty externally. No evident gas infection.

9.0 a.m. 10 c.c serum (W.G. 22) intramuscularly.
2.0 p.m. T. 102·4 P. 120 R. 28.
6.0 p.m. T. 97·8 P. 104 R. 26.
10 p.m. T. 100 P. 106 R. 30.

For two days following this the patient was in about the same condition, the morning temperature each day being down to normal and the evening temperature up to 104, with P. 110 to 140. No evidence of local gas infection spreading or of gas intoxication. Looks like a streptococcal infection.

9. 5. 18. General condition the same or better.
a.m. T. 98, P. 108, R. 36. p.m. T. 101, P. 120, R. 32.
10. 5. 18. 6 a.m. T. 101·2, P. 116, R. 30.
12 noon T. 97·8, P. 112, R. 32.

Patient had 150 c.c. serum in first 10 hours from commencement of treatment. In the following 24 hours he had 40 c.c., and in the next 24 hours, 90 c.c., i. e. 280 c.c. in all. Considering the extensive gas gangrene of the testicle and cord, its extension as far as the renal vessels, the size of the wound made in removal and the patient's general poor condition, it seems reasonable to ascribe his improvement in large part to the serum. At the time of the second operation the gas infection was well established and there was considerable infection in the retroperitoneal tissues of the iliac fossa. He was considered clinically an almost hopeless case. He now has a tremendous open wound, which when he lies on his opposite side exposes the muscular wall of the abdominal cavity from the bottom of the pelvis up to the lower pole of the kidney. The surfaces of this are covered with necrotic exudate but there is no evidence of deep infection. For the past week he has had the appearance of a streptococcal infection, but the last two days has definitely improved and now appears to have a fair chance of recovery. Since the first day's serum treatment he has had nothing to suggest spread of gas infection or intoxication. The morning after the second operation the patient had the appearance of gas intoxication and in the opinion of his surgeon was a true gas intoxication.

(iv) *Discussion.*

The serum therapy of gas gangrene has presented a problem of very great complexity. The reasons for this may be thus briefly outlined.

1. Gas gangrene is a polymicrobic disease. The chief organisms responsible for the condition are, in the order of their frequence and of their relative importance, *B. welchii, vibrion septique, B. oedematiens,* and *B. fallax.* Each of these organisms, either by itself or in combination with the others, has been found to be the principal pathogenic agent in fatal human cases. In addition, however, to these arch-criminals in the bacteriological syndrome, there are a certain number of anaerobes and of aerobes, non-pathogenic in themselves, which act as aiders and abettors in the production of infection. Not only is the number of possible bacterial combinations enormous, but it is also impossible for the bacteriologist, except after prolonged investigation, to determine the special combination of organisms which is responsible for the disease in any individual case. It follows therefore that, whereas in the case of diphtheria and tetanus we have to deal with a specific and well-defined entity, in the case of gas gangrene we are faced with a disease which is much more complicated, so that a successful therapy can be undertaken only with a serum that is polyvalent. The requisite constituents of such a serum are dealt with in the summary which concludes this report.

2. The nature of the disease is such that it may be difficult to introduce the serum into the general circulation or to bring it into contact with the infected tissues.

(*a*) The constriction of the peripheral veins, which is a marked feature in certain cases of gas gangrene toxaemia may render it difficult to carry out intravenous inoculation. Also, the depressed state of the circulation reduces to a minimum the chance of absorption when the serum is given subcutaneously or intramuscularly.

(*b*) The infected tissues may be cut off from the general circulation owing to mechanical damage or occlusion of the vessels resulting from the wound itself. Or again, the vascular supply may be seriously reduced because of the oedema which is such a characteristic feature of the pathological process.

The fortunate results reported by Ellis, were, we believe, due in large measure to the inoculation of serum into and around infected tissues.

The short incubation period in man, and the ruthless rapidity with which the disease progresses when it is once established, are further factors which militate against successful treatment with serum.

3. Of the anaerobes which produce gas gangrene in man, *B. oedematiens* is the only organism which yields a toxin comparable in valency to that given by *B. diphtheriae* or *B. tetani.* The toxins of *B. welchii* and of *vibrion septique,* when tested on laboratory animals, are of extremely low value in comparison with those just mentioned.

It may be accepted as a general principle in the immunization of horses against toxins that the titre of the resulting antiserum varies directly with the value in toxin or toxoid of the material inoculated. The problem therefore of producing better value *B. welchii* or *vibrion septique* sera is identical with the problem of producing better toxin.

(v) *Summary.*

1. Our information in regard to diphtheria and tetanus is based on countless laboratory experiments and a vast clinical experience extending over three decades. In contrast to this, our knowledge of the anaerobes is limited to investigations which have extended over a few months and which were frequently undertaken in unfavourable conditions. In this respect the work on anaerobes may be said to be still in its infancy, so that the serum therapy of the disease, as practised in the last stages of the war, was no more than an experiment.

In view of the numerous factors which militate against success and which have been dealt within various sections of this report we consider that the results obtained in France have exceeded expectations.

2. The sera which were issued for use in British hospitals in France contained antitoxin to *B. welchii* and *vibrion septique* only. Laboratory experiments have shown that it is necessary to include antitoxin to *B. oedematiens* and *B. fallax* in addition, and the clinical reports from France add corroborative evidence to this finding.

The value of antibacterial substances in a gas gangrene serum is as yet not clearly established.

3. A gas gangrene serum should be given at the earliest possible stage in the illness. It is probable that prophylactic inoculations, if practised with sufficiently large doses, would give results which cannot be obtained when the serum is used therapeutically to check the already established disease.

The production of an active immunity in man by the inoculation of carefully adjusted toxin-antitoxin mixtures is suggested as a procedure which might considerably curtail the incidence of the disease and diminish its morbidity rate.

(vi) *Table of Cases.*

Abbreviations, etc., used in the table giving details of cases treated with serum.

Figures inserted in brackets in front of letterpress in the columns headed ' Surgery ' ' Serum ', ' Result ', and ' Bacteriology ' indicate *days* after admission.

In the columns headed ' Serum ' the figures following on those in brackets indicate the number of cubic centimetres of serum given. The letters I.V., I.M., S.C. indicate intravenous, intramuscular, or subcutaneous. A letter followed by a number indicates the batch of serum employed.

For instance (6) 50 I. V. G17 indicates that on the sixth day after admission 50 c.c. of serum were given intravenously, the batch of serum being G17.

Other abbreviations occurring in this column are A.T.S. which indicates antitetanic serum, and when this is followed by a number, e. g. 750, it gives the number of units in the dose administered A.T.S. +W indicates antitetanic serum with the addition of antiwelchii serum.

In the column headed ' Result ' the sign + indicates the death of the patient, the figures in brackets, as mentioned above, giving the number of days after his admission to hospital ; the sign — indicates that the patient recovered.

Case.	*Reported by.*	*Lesion.*	*Day of onset of gas gangrene.*	*Surgery*
1. Fe.	Barling	Right leg and left thigh.	2	(1) Right leg cleaned up. (2) Left thigh cleaned up, removal of foreign body and bits of clothing.
2. Pi.	Barling	Left shoulder and left foot.	2	(2) Foot amputated, foreign bodies removed from shoulder. (6) Multiple incisions left arm and shoulder.
3. Jo.	Barling	Left thigh.	2	(1) Femoral vessels tied. (2) Amputation and excision of muscle. (7) Excision of part of femoral vein, necrosed muscle removed.
4. Ey.	Barling	Left thigh, left shoulder, right arm.	1	(1) Wound cleaned, foreign body removed. (4) Left hip explored. (8) Left thigh opened up. (11) Amputation left arm.
5. Co.	Ellis	Right thigh.	1	(1) Excision of wound and infected muscle.
6. Ch.	Ellis	Buttock.	1	(1) Foreign body removed and infected muscle removed.
7. Sm.	Ellis	Thigh and hand.	1	(1) Amputation.
8. McI.	Ellis	Thigh and thorax.	1	(1) Amputation.
9. Rh.	Ellis	Multiple wounds thigh and leg.	1	(1) Amputation, recurrence in stump.
10. Bo.	Ellis	Left thigh and right leg.	1	(1) Amputation.
11. Co.	Ellis	Right knee.	1	(1) Amputation.
12. Le.	Ellis	Right leg and right arm.	1	(1) Excision of wound and muscle.
13. St.	Ellis	Both legs and thighs.	2	(2) Amputation.
14. Sa.	Hope	Neck and shoulder	13	(1) Excision of wounds, thyro-hyoid membrane sutured. (13) Ribs excised, stinking haemothorax drained.

Serum.	*Result.*	*Bacteriology.*	*Remarks.*
20 I.V. G 3.	(6) +	.. ..	Much relieved by serum; it ought to have been repeated.
50 I.V. G 17.	(7) +	*B. welchii*, *B. sporogenes*, and streptococci.	Death 7 hours after serum.
40 I.M. G 3.	(7) +	.. ..	Serum had no effect on general or local condition.
60 I.V. G 17 0 I.V. G 22.	(11) +	.. ..	Death a few hours after operation and the giving of serum.
60 I.M. G 53.	—		
60 I.M. G 53. 60 I.M. G 53.	—	Wound gave *B. welchii* and streptococci.	
60 I.M. G 53. 10 I.V. G 53.	—		
80 I.M. G 53. 40 I.M. G 53. 40 I.M. G 53. 40 I.M. G 53.	—	(2) Thigh gave *B. welchii*, *B. sporogenes*, and streptococci. (2) Haemothorax gave *B. welchii*, *B. sporogenes* and streptococci. (7) Haemothorax gave *B. welchii* and streptococci.	Cured by serum. Fatal prognosis given by surgeon.
80 I.M. G 53. 20 I.M. G 53.	—	Muscle gave *B. welchii*.	Cure following serum therapy in spite of fatal prognosis.
100 I.V. G 53.	(3) +	(3) Blood gave *B. welchii*, *B. sporogenes* and streptococci.	Death 6 hours after serum.
60 I.M. G 53.	(2) +	.. ..	Death 4 hours after serum.
100 I.V. G 53. 100 I.V. G 53.	(2) +	Blood culture gave a sporing anaerobe. Muscle gave the same anaerobe together with *B. sporogenes*.	Antitoxic sera prepared against *B. welchii*, *vibrion septique*, and *B. oedematiens* did not protect guinea-pigs against this anaerobe. (Henry). Quoted in full in Section VI of report.
100 I.V. G 53. 30 I.M. G 53. 60 I.M. 80 I.M. 50 I.M. 60 I.M.	—	Muscle gave *B. welchii*, *B. sporogenes*, and streptococci.	A welchii-septique antitoxic serum protected a guinea-pig against the muscle culture (Henry). Quoted in full in Section VI of Report.
A.T.S. 500. A.T.S. 750. 50 I.V. G 17.	(16) +	.. ..	Anaphylactic death.

Case.	*Reported by.*	*Lesion.*	*Day of onset of gas gangrene.*	*Surgery.*
15. Sto.	McEwen	Thigh.	2	(2) Ligature of femoral ve excision of muscle. (3) Amputation.
16. Ta.	McNee	Right shoulder and arm.	1	(1) Wound cleaned and mu cised.
17. Li	McNee	Both thighs, feet, wrist, &c.	1	(1) Wounds cleaned.
18. McL.	McNee	Right forearm.	1	(1) Wound cleaned. (2) Amputation.
19. Co.	McNee	Thorax.	..	(1) Aspiration. (3) Rib resection.
20. Ho.	McNee	Abdomen and both thighs.	1	(1) Wounds cleaned. (3) Further operation.
21. McD.	McNee	Shoulder, back and both buttocks.	1	(1) Shoulder, disarticulation.
22. Co.	McNee	Arm and thigh.	2	(1) Wounds cleaned. (2) Amputation.
23. Ev.	McNee	Left arm.	1	(1) Infected muscle removed.
24. Eck.	McNee	Left knee and ankle.	1	(1) Wound cleaned and in muscle removed.
25. Do.	McNee	Knee.	2 (stump)	(1) Amputation.
26. Ea.	McNee	Leg, posterior tibial vessels torn.	..	Wounds cleaned up, ampu later.
27. Fr.	McNee	Left arm.	1	(1) Excision of muscle.
28. Da.	McNee	Right thigh, left hand and back, left shoulder.	1	(1) Excision of muscle.
29. Ki.	McNee	Right leg and thigh.	1	(1) Excision of wound.
30. Pe.	McNee	Right buttock.	1	(1) Cleaning of wound and ex of muscle. (2) Further excision.
31. Cu.	McNee	Thigh and leg.	1	(1) Excision of muscle.
32. Ro.	McNee	Arm, brachial artery divided.	3	(1) Wound cleaned up. (3) Amputation.
33. Ha.	McNee	Thigh and leg.	1	(1) Excision of muscle. (3) Amputation.
34. An.	McNee	Right leg.	1	(1) Amputation through knee.

Serum.	*Result.*	*Bacteriology.*	*Remarks.*
12) 10 I.M. G 19.	+	.. ..	? Anaphylactic death.
(1) 25 B. 1400.	(2) +		
(1) 30 G 3.	(1) +	.. ..	Death few hours after serum.
(1) 20 B. 725.	—		
(3) 40 B. 1100. 40 B. 1100.	(5) +	Haemothorax fluid gave pure *B. welchii.*	
(1) 20 B. 1175. (3) 20 G 2. 40 G 2.	(4) +		
(1) 50 B. 1175. (2) 20 B. 1175. (3) 20 B. 1175.	(3) +	P. M. oedema fluid gave *B. welchii, B. sporogenes,* and ? *B. oedematiens.*	
(2) 30 B. 1175. (3) 40 B. 1175.	—		
(1) 40 B. 1400. (3) 40 B. 1400.	—		
(1) 40 G 3. (1) 40 G 3.	—		
(2) 40 G 2.	—		
20 B.	—		
(1) 50 G 2. (2) 30 G 2.	—		
(1) 40 B. 1175. (2) 100 G 3.	(3) +		
(1) A.T.S.+ W. (2) 20 B.	—		
(1) 50 G 2. (2) 50 G 3.	—		
(1) 50 B. 1175.	+	.. ..	Died 9 hours after operation and serum.
(1) A.T.S.+ W.	—	.. ..	Small prophylactic dose of serum only given.
(3) 80 B. 725. (4) 80 B. 1400.	+	.. ..	A welchii-septique antitoxic serum protected mice against whole culture (Henry).
(1) A.T.S.+ W. (1) 40 G 2.	—		

Case.	*Reported by.*	*Lesion.*	*Day of onset of gas gangrene.*	*Surgery.*
Sa.	McNee	Thigh and elsewhere.	1	(1) Amputation of thigh and excisi of other wounds.
Bo.	McNee	Multiple.	1 (Thigh)	(1) Amputation left leg.
Pi.	McNee	Right arm.	1	(1) Wounds cleaned up.
Th.	McNee	Multiple.	3	(3) Amputation right arm and l leg; other wounds excis and foreign bodies remove
Wa.	McNee	Left thigh.	1	(1) Amputation.
Wo.	McNee	Left leg.	1	(1) Amputation.
Sm.	McNee	Left arm, brachial artery and median nerve torn.	2	(1) Wound cleaned, artery tied, a nerve sutured. (2) Amputation.
Ma.	McNee	Arm.	1	(1) Disarticulation at the shoulde
Po.	Stokes	Thigh and buttock gluteal artery torn, sciatic nerve damaged, fractured pelvis, rectum torn.	2	(1) Wounds excised, foreign bo removed.
Cr.	Stokes	Leg.	1	(2) Circular amputation, some n crosed muscle left.
We.	Stokes	Arm, trunk, and right leg.	2	(1) Partial amputation at field a bulance. (3) Amputation.
Br.	Stokes	Thigh and pelvis.	1	Too ill for operation; forei bodies and bits of bone r moved.
Ba.	Stokes	Arm and thorax.	1	(1) Wound cleaned up and haem thorax aspirated.
Mo.	Stokes	Thigh.	1	Inoperable, pulseless, and delirio on admission.
Po.	Stokes	Thorax.	1	.. ..
Re.	Stokes	Knee and thigh.	1	(3) Amputation.

Serum	*Result.*	*Bacteriology.*	*Remarks.*
1) 20 I.V. B. 2) 30 I.V. B. 3) 30 I.V. B.	—		
2) 20 I.V. B. 2) 30 I.V. B. 3) 30 I.V. B.	—		
1) 20 B. 2) 30 B.	—		
3) 20 B. 4) 30 B. 6) 30 B.	(10) +	.. ..	Death from lobar pneumonia.
1) 30 B.	+	.. ..	Death 10 hours after operation.
1) 20 I.V. B.	—		
2) 30 B.	—		
1) 30 B.	—		
2) 40 I.V. G 18 and G 19.	+	Blood culture gave streptococcus. Wounds gave *B. welchii*, *B. sporogenes*, and streptococci.	Death 11 hours after operation.
2) 30 I.V. G 19. 2) 40 I.V. G 19. 3) 50 I.V. G 19.	—	Blood culture sterile. Wound gave *B. welchii*, *B. sporogenes*, another welchii-like anaerobe and streptococci.	
4) 20 I.V. G 19. 5) 20 I.V. G 19.	(10) +	Wound gave *B. welchii*, streptococci, and staphylococci.	Death due to lobar pneumonia.
) 20 I.V. G 19. 30 S.C. G 19. 10 locally.	(3) +	(1) Blood culture nil. (1) Wound gave *vibrion septique* and *B. welchii*. (2) P.M. heart blood gave *vibrion septique* and *B. welchii*.	
) 20 I.V. G 18. 30 S.C. G 18.	—	(1) Blood culture nil. (1) Wound gave *B. welchii*, *B. fallax*, and *B. sporogenes*.	
) 60 I.V. G 3 and G 22.	(2) +	(1) Blood culture gave pure *B. fallax*. (1) Blister fluid gave *B. fallax* and *B. welchii*.	Died 1 hour after serum.
) 30 I.V. G 17.	(3) +	Haemothorax fluid gave pure culture of *B. welchii*.	Died 6 hours after serum.
) 50 S.C. G 18.	—	(3) Blood culture nil; wound gave *B. welchii*, *B. sporogenes*, and another anaerobe not identified.	

Case.	*Reported by.*	*Lesion.*	*Day of onset of gas gangrene.*	*Surgery.*
. Fr.	Stokes	Right thigh, left thigh, and right groin.	1	(1) Wounds cleaned, femoral v tied. (12) Amputation for streptococ infection of knee.
. To.	Stokes	Multiple wounds both legs and right arm.	2	(2) Wounds excised and forei bodies removed.
. Fo.	Stokes	Both thighs and right arm.	1	(1) Wounds cleaned and forei bodies removed. (2) Amputation of left leg.
. Sa.	Stokes	Thigh.	1	(1) Multiple incisions. (2) Amputation.
. Br.	Stokes	Leg and thorax.	1	(1) Wounds cleaned up. (2) Excision of infected muscle.
. Du.	Stokes	Arm and head.	1	(1) Amputation of arm. (2) Head operation.
. Co.	Stokes	Left femur and right thigh.	2	(1) Amputation left thigh. (2) Excision muscles right thigh.
. Sy.	Stokes	Thigh.	2	(1) Excision of wound and ligatu of femoral vein. (2) Amputation through thigh.
. Wa.	Stokes	Multiple wounds of both thighs.	1	No operation.
. Ca.	Stokes	Thigh.	1	(1) Excision of infected muscle ar removal of foreign body.
Ha.	Stokes	Buttock.	3	(1) Excision of muscle and remov of foreign body. (2) Further excision of muscle.
Ha.	Stokes	Buttock.	2	(1) Foreign body removed and wound track excised.

Serum	*Result.*	*Bacteriology.*	*Remarks.*
35 I.V. G 19. 15 S.C. G 19.	—	Blood culture nil; wound gave *B. welchii*, *B. sporogenes*, and another anaerobe not identified.	
(3) 25 I.V. G 19. 25 S.C. G 19.	—	Blood culture gave *B. welchii* and *B. sporogenes*; wound gave *B. welchii*, *B. sporogenes*, streptococci, diphtheroids, and coliforms.	
(1) 20 I.V. G 19. 30 S.C. G 19. (3) 40 S.C. G 19.	—	(2) Blood culture gave *B. welchii*. (3) Blood culture negative. (2) Wound gave *B. welchii* and other anaerobes.	
(1) 30 I.V. G 17. 30 I.V. G 22. (2) 90 I.V. G 17 and G 22.	(3) +	(1) Blood culture gave *B. fallax*. (2) Blood culture gave *B. fallax*. (3) Blood culture negative. (1) Oedema fluid gave *B. welchii* and *B. fallax*.	
(2) 60 I.V. G 19.	(2) +	(2) Blood culture gave *B. welchii*.	Restless, pulseless, and pallid 2 hours after serum. Death 7 hours after serum.
(2) 40 S.C. G 22.	+	Cultures from the arm gave *B. sporogenes*, *B. welchii*, and proteus. Cultures from head gave *B. welchii* and *B. sporogenes*.	Late death from meningitis. Streptococci and staphylococci only found in cultures made from the meningeal exudate.
(2) 60 I.V. G 22.	(2) +	(2) Blood culture negative. (2) Heart blood taken at *post mortem* gave *B. welchii*. Cultures from wound gave *B. welchii*, *B. sporogenes*, streptococci, and a coliform bacillus.	Death 5 hours after serum.
(2) 60 I.V. G 44.	(2) +	(2) Blood culture negative; *B. welchii* and *B. sporogenes* isolated from the wound.	Death 2 hours after serum.
(1) 60 I.V. G 44. (2) 60 I.V. G 44.	(2) +	(1) Cultures from the wound gave *B. welchii*, *B. sporogenes*, and a non-pathogenic tetanus-like bacillus.	
(1) 80 I.V. G 22. 40 S.C. G 22.	(2) +	(1) Blood culture negative. (2) Post-mortem heart blood gave *B. welchii* and streptococci; the wound yielded *B. welchii*, *B. sporogenes*, and streptococci.	
(3) 60 I.V. G 22. 60 S.C. G 22.	—	Cultures from wound gave *B. welchii* and *B. sporogenes*.	Gas gangrene arrested by serum
(2) 60 I.V. G 22.	(2) +	(1) Blood culture gave an aberrant *B. welchii*. Wound cultures gave this aberrant bacillus, *B. welchii* and *B. sporogenes*.	

Case.	Reported by.	Lesion.	Day of onset of gas gangrene.	Surgery.
Ga.	Stokes	Lumbar region.	1	(1) Excision of infected muscle.
Cla.	Stokes	Back.	1	(1) Excision of muscles. (2) Further excision.
Sa.	Stokes	Thigh.	1	No operation.
Wa.	Tytler	Left thigh.	2	(2) Amputation of leg. (5) Transfusion with blood.
McK.	Tytler	Right forearm.	2	(2) Excision of infected muscles.
Jo.	Tytler	Both legs.	2	(2) Amputation left thigh.
Bis.	Tytler	Right thigh.	6	(2) Wound excised. (6) Thigh incised.
Ha.	Tytler	Right thorax.	4	(5) Bullet removed from back, haemothorax fluid aspirated.
Jo.	Tytler	Left leg and shoulder.	2	(2) Amputation. (5) Excision of muscle from stump and buttock.
Pi.	Tytler	Right thigh and buttock, right arm, and head.	3	(2) Excision of wounds and removal of foreign bodies. (3) Excision of buttock muscles.
Il.	Tytler	Head.	2	(2) Wound excised, skull trephined, foreign body removed.
Jar.	Tytler	Left shoulder and back.	..	Extensive muscle excision.
Col.	Tytler	Thorax.	..	Aspiration followed by rib resection.

Serum.	*Result.*	*Bacteriology.*	*Remarks.*
1) 60 I.V. G 22.	(9) +	*B. welchii* and two types of *B. sporogenes* isolated from the wound.	Gas gangrene arrested by serum; death from streptococcal septicaemia.
2) 60 I.V. G 22. 40 S.C. G 22.	(2) +	(2) Blood culture gave *B. welchii* and streptococci.	Death a few hours after serum.
2) 60 I.V. G 22.	(2) +	.. ..	Died 8 hours after serum.
5) 10 I.V. G 3.	—	Wound gave *B. welchii* and streptococci.	
4) 20 I.V. G 17. 20 I.M. G 17.	—	(4) Blood culture negative; wound gave pure *B. welchii.*	
5) 20 I.V. G 22. 20 I.M. G 22.	(5) +	Wound gave *B. welchii* and streptococci.	Death 4 hours after serum.
8) 20 I.V. G 22.	(8) +	Wound gave *B. welchii* and streptococci.	Death ½ hour after serum.
6) 20 I.V. G 22. 40 I.M. G 22.	(6) +	Haemothorax fluid gave *B. welchii.* Heart blood *post mortem* gave *B. welchii.*	Death 6 hours after serum.
5) 20 I.M. G 17. 6) 20 I.M. G 17.	—	Smear from wound showed *B. welchii,* but cultures were negative.	
3) 20 I.V. G 22. 20 I.M. G 22.	(3) +	(3) Blood culture gave *B. welchii* and a streptococcus. Wound at *post mortem* gave *B. welchii* and a streptococcus.	Death 3 hours after serum.
2) 20 I.M. G 19. 3) 40 I.M. G 19. 4) 20 I.M. G 19. 20 I.M. G 22. 5) 40 I.M. G 22. 6) 40 I.M. G 22. 7) 40 I.M. G 22.	(8) +	(2) Wound gave *B. welchii.* (4) Wound gave *B. welchii* and streptococci. (6) Wound gave *B. welchii* and streptococci. Heart blood *post mortem* gave *B. welchii.*	
3) 10 I.V. G 3. 20 S.C. G 3. 20 S.C. G 3. 4) 20 S.C. G 3.	(6) +	(3) Muscle excised at operation gave *B. welchii* and streptococci. (4) Wound gave *B. welchii,* streptococci, and some spore-bearing bacilli. (6) Heart blood *post mortem* gave *B. welchii* and a haemolytic streptococcus. Post-mortem cultures from pericardial fluid and liver yielded pure cultures of haemolytic streptococci.	Death probably resulted from streptococcal septicaemia.
2) 20 I.V. G 22. 20 I.V. G 22. 20 I.V. G 22. 10 I.V. G 22. 10 I.V. G 22. 10 I.V. G 22. Total 90 c.c. in 24 hours.	(14) +		

Case.	*Reported by.*	*Lesion.*	*Day of onset of gas gangrene.*	*Surgery.*
Ke.	Tytler	Left back.	3	(3) Wound excised and large forei body removed from pso muscle.
Ol.	Wyard	Back, neck, and tongue.	4	(1) Wounds excised and cleaned.
Con.	Wyard	Left buttock, groin, and pelvis.	2	.. ..
Qu.	McNee	Buttock.	1	(1) Wound laid open and infecte muscle excised.
C.	McNee	Right elbow; brachial artery and veins divided.	2	(1) Wounds cleaned; vessels tied ulnar nerve sutured. (2) Amputation.
Pu.	McNee	Thigh.	2	(1) Excision of muscle. (2) Excision of muscle.
Cu.	McNee	Both thighs; also chest with haemothorax.	1	.. ..
Pro.	Brenan	Haemothorax.	2	(2) Resection of rib; evacuation of haemothorax fluid; removal of foreign body and bits of clothing from the lung.
Ap.	Tytler	Left buttock.	..	.. ..
mi.	Tytler	Multiple wounds.	2	(1) Amputation right thigh. (2) Excision wound left thigh.

Serum.	*Result.*	*Bacteriology.*	*Remarks.*
) 20 I.V. G 22. 120 I.M. G 22.) 40 I.M. G 22.) 90 I.M. G 22.	—	Culture from gangrenous testicle gave *B. welchii* and streptococci.	Gas gangrene checked by serum. Case reported in full in Section VI.
) 40 I.V. G 18. 60 I.V. G 19.) 20 I.V. G 19.	—	Cultures from wound gave no growth of anaerobes.	
) 20 I.M. G 17.) 100 I.V. G 17.	(8) +	Muscle from *post mortem* showed ? *vibrion septique.*	
) A.T.S+ W.) G 44.	(3) +	Cultures from muscle gave *B. welchii, B. sporogenes,* streptococci, and another anaerobe. A welchii-septique antitoxic serum did not protect a guinea-pig against the mixed muscle culture (Henry).	
40 B.	—	Cultures from muscle gave *B. welchii, B. sporogenes,* a round end-sporer, and streptococci. A welchii-septique antitoxic serum protected a guinea-pig against the mixed muscle culture (Henry).	
) A.T.S+ W.) 20 G 44.	..	Cultures from muscle gave *B. welchii, B. sporogenes,* a round end-sporer, an oval end-sporer, and another anaerobe. A welchii-septique antitoxic serum protected a guinea-pig against the mixed muscle culture (Henry).	
) 20 G 44.	(2) +	Cultures from muscle gave *B. welchii, B. sporogenes,* a round end-sporer, an oval end-sporer, another anaerobe, streptococci, and a coliform bacillus. A welchii-septique antitoxic serum protected a guinea-pig against the mixed muscle culture (Henry).	
30 B.	(5) +	Cultures from haemothorax fluid gave *B. welchii* and *B. sporogenes.* A welchii-septique antitoxic serum protected a guinea-pig against this mixed culture (Henry).	
) 100 I.V. G 44.	(14) +	Culture from infected muscle gave *B. welchii* and *B. oedematiens.* A welchii-septique antitoxic serum did not protect a guinea-pig against this mixed culture (Henry).	
5) 30 I.M. G 22. 6) 30 I.M. G 22. 6) 20 I.V. G 53.	(6) +	Culture from infected muscle gave *B. welchii,* streptococci, and an unidentified anaerobe. A welchii-septique antitoxic serum protected a guinea-pig against this mixed culture (Henry).	

Case.	*Reported by.*	*Lesion.*	*Day of onset of gas gangrene.*	*Surgery.*
). She.	Tytler	Multiple, arm.	3	(3) Amputation.
'. Du.	Tytler	Thigh.	2	(2) Wounds cleaned up.
'. Coo.	Tytler	Buttock, torn gluteal artery.	2	(1) Wound cleaned, gluteal arte tied.
. Me.	Tytler	Thigh and buttocks.	1	(1) Wounds cleaned. (4) Excision of hamstring muscle

Serum.	*Result.*	*Bacteriology.*	*Remarks.*
3) 50 I.M. G 22. 3) 20 I.V. G 22.	—	Culture from infected muscle gave *B. welchii* and streptococci. A welchii-septique serum protected a guinea-pig against this mixed culture (Henry).	
2) 100 I.M. G 44.	—	Cultures of material from the wound gave *B. welchii*, streptococci, and an unidentified anaerobe. A welchii-septique serum protected a guinea-pig against this mixed culture (Henry).	
7) 20 I.V. G 22. 7) 50 I.M. G 22. 3) 90 I.M. G 22.)) 20 I.M. G 22. [) 20 I.M. G 22. 2) 20 I.M. G 22.	(21) +	(5) Blood culture gave *B. welchii* and streptococci. (9) Blood culture gave streptococci only.	
4) 100 I.M. G 22 7) 80 I.M. G 44. 3) 40 I.M. G 44.	(9) +	A welchii-septique serum did not protect guinea-pigs against mixed muscle cultures from this case; nor did a high-titre oedematiens serum. An organism was obtained in pure culture from the heart blood of guinea-pigs which had succumbed to infection by mixed muscle cultures. This organism is believed to be a pathogenic *B. fallax* (Henry).	

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APPENDIX

THE HISTOPATHOLOGY OF GAS GANGRENE

By E. H. Kettle, M.D. Lond.

A systematic study of the histopathology of any infection should proceed along certain definite lines, and should include investigations into

1. The distribution and dissemination of the organisms in the body.

2. The changes taking place at the site of infection.

3. The lesions produced in the other tissues and organs of the body.

A. From the action of circulating toxins formed by the organisms.

B. As the result of the presence of the organisms.

4. Reactions taking place in various organs in the form of—

A. Attempts to combat the infection.

B. Changes accompanying the process of healing and repair.

Although the anaerobic bacilli do not lend themselves very readily to investigation along these lines, I have adopted this scheme as a working basis since it is desirable to bring these organisms into comparison with other pathogenic microbes.

For several reasons, the material derived from cases of gas-gangrene in the human being are often unsatisfactory. The lesions due to the presence of anaerobic bacilli in the body are essentially degenerative, and it may be impossible to distinguish them from similar changes due to other causes; and another source of confusion is the *post-mortem* degeneration which must elapse between the death of the patient and the autopsy. Further, there is frequently a coexisting infection with other organisms which again complicates the analysis of the histological findings. The human material to which I had access suffers in a varying degree from these defects, and, in addition, the greater part of it was quite uncontrolled by clinical or bacteriological data.

The lack of satisfactory human material may be compensated for to some extent by the use of laboratory animals. Rabbits, guinea-pigs, rats and mice, can all be infected by subcutaneous or intramuscular inoculation, but it is not easy to reproduce the disease as it occurs in man. Either the dose is too small and the inoculations fail to take effect, or the local lesion is so severe that the animals die rapidly from toxaemia before the disease becomes generalized. Similarly, I have found that intravenous inoculation with cultures

of the bacilli produce death without the formation of localized lesions. Of the experimental material which had been placed at my disposal, very little was of value, and, under these circumstances, this investigation is incomplete in many particulars. In respect to section 4 of the scheme my observations are insufficient to admit of conclusions being drawn, and I shall therefore omit any discussion of this part of the problem.

1. *The Distribution and Dissemination of the organisms in the body.*

The severity and extent of the lesions at the site of inoculation have so dominated the clinical picture that gas-gangrene has come to be looked upon as a local disease of muscle. This, however, is not correct. The organisms are not confined to the neighbourhood of the wound but are distributed widely throughout the body, producing lesions which are identical with those occurring in muscle. It must be recognized, of course, that the bacilli may proliferate extensively in the body after death, and that their distribution in tissues obtained at autopsy is not always representative of their ante-mortem dissemination. But when due allowance is made for this, an examination of human and experimental material shows that septicaemia is of common occurrence.

In human material, organisms have been found in association with definite lesions in the heart muscle, the liver, the kidney, the spleen, the lymph glands, the supra-renal glands, and the meninges of the brain. Apart from lesions, I have found organisms widely distributed in veins and capillaries. In many instances this dissemination must be regarded as a post-mortem phenomenon, but cases remain in which the only possible explanation is a terminal septicaemia.

In animals, probably because of the rapidity with which they succumb to toxaemia, septicaemia is uncommon. I have, however, been able to demonstrate organisms in the circulating blood and in the bone marrow of animals killed at varying times after intramuscular inoculation.

Dissemination of the organisms occurs in three ways:—

(*a*) By direct extension in loose areolar and connective tissue.

(*b*) By growth along lymphatic vessels.

(*c*) By invasion of the blood-stream.

(*a*) *Dissemination by direct extension in loose areolar and connective tissue.* This is probably the most important mode of dissemination since it is the way in which infection spreads from the primary wound till the whole limbs and even the trunk become involved. The muscles are only of importance in defining the path of infection inasmuch as the areas enclosed by the epimysium and its prolongations, the perimysium, form, for all practical purposes, lymphatic channels in which the organisms can advance. The bacilli flourish in loose areolar tissue and tend to spread along tissue spaces and connective tissue planes with great rapidity.

(*b*) *Dissemination by growth along lymphatic vessels.* The bacilli grow very readily within the lymphatic vessels as distinct from the

tissue lymph spaces, a condition of permeation occurring which is very similar to that seen in the spread of a malignant growth.

Lymphatic permeation by the bacilli can be demonstrated in the neighbourhood of the site of inoculation in animals, and I have also found it taking place in human material. A particularly striking instance was seen in a deep cervical gland of a child who died of gas-gangrene of the arm. Areas of the gland showed early localized degenerative changes, and a main afferent lymphatic vessel was plugged with a thick column of bacilli.

(*c*) *Dissemination by invasion of the blood-stream.* This is not so easy to demonstrate. Occasionally, in the local lesion, a few organisms can be detected in the capillaries or veins, but this invasion of the blood-stream is quite overshadowed by the luxuriant growth of the organisms in the tissues and the lymphatics. Nevertheless, it would appear that visceral lesions are due to a blood-borne infection. Bacilli may frequently be demonstrated in the sinusoids of the liver, even though they may be absent in other organs; and I have found them in considerable numbers in the capillaries of the glomeruli. I have never, however, found bacilli in the lungs under conditions in which post-mortem change could be excluded; and I have never seen pulmonary lesions which could be ascribed to them.

2. *The changes taking place at the site of infection.*

The changes in muscle following infection with anaerobic bacilli have been fully described, and it is unnecessary to deal at any length with this aspect of the question. It is important, however, to realize that the infection of muscles is purely an accidental phenomenon. Anaerobic bacilli have no specific action on muscles, nor do they find in them any substances which are especially necessary to their metabolism. It is in deep penetrating wounds that the organisms thrive best, and in the limbs such wounds involve muscle. Wounds of the thoracic or abdominal viscera are fatal, or receive operative treatment, from considerations other than the occurrence of gas-gangrene, but were these organs of less immediate importance to the life of the individual there is no reason to suppose that the incidence of gas-gangrene would not be as high in them as it is in wounds of muscle.

The characteristic lesion of gas-gangrene is necrosis brought about by the toxins elaborated by the organisms. The changes are most obvious in the muscle fibres which pass through the stages of cloudy swelling, and gradual loss of striation, to coagulation necrosis and solution. The most highly organized structures, the muscles, nerves, and the epidermis and its derivatives are affected earliest, but eventually the blood-vessels and the connective tissues are also destroyed. The organisms, which are usually present in enormous numbers, lie in the connective tissues, and do not invade the other structures except, perhaps when necrosis is far advanced. In an infected muscle they tend to remain within the limits of the epimysium; and they extend to neighbouring muscles by spreading in the subcutaneous tissue, or the deep connective tissue trabeculae.

The most striking feature of the lesion is the entire lack of any inflammatory reaction. Some proliferation of the sarcolemma nuclei can sometimes be seen in front of the advancing margin of the infection, but this is all. The muscle fibres are absolutely quiescent, and, moreover, there is a complete absence of wandering cells. In the subcutaneous and connective tissues, on the other hand, there occurs a leucocytosis which may be very pronounced; the leucocytes are of the polymorphonuclear variety, and are actively phagocytic. There is little or no reaction of the fixed connective tissue cells.

The blood-vessels show no very characteristic lesions. In advanced cases the constituents of their walls lose their staining reactions and undergo necrosis in common with the rest of the tissues, but in the early stages they present no constant changes.

Thrombosis of veins and capillaries frequently occurs but is not invariable. I have not been able to detect any alteration in the endothelium to account for this, but there is clear evidence of a toxic destruction of red blood corpuscles which in itself would tend to cause conglutination thrombi. In advanced lesions haemorrhage and haemolysis frequently occurs.

I have not been able to come to definite conclusions as to possible differences in the lesions produced by the various anaerobic bacilli. *B. oedematiens* and *V. septique* appear to give rise to greater oedema and more advanced vascular changes than *B. welchii*, and haemorrhage and thrombosis appear to be particularly associated with *V. septique*, but my observations on this point are not conclusive.

3. *The lesions produced in the other organs and tissues of the body.*
A. From the action of circulating toxins.

The finer details of cell degeneration are often obscured by post-mortem changes; experimental material is thus particularly necessary for this portion of the investigation. By comparing the tissues of animals, killed some twenty-four hours after being infected with cultures of anaerobic bacilli, with human organs obtained at autopsy, it is possible to arrive at some idea of the changes which usually take place. It was my intention to work also with animals which had been inoculated with toxins alone, but I was forced to abandon this line of research since most of the material at my disposal proved to be infected with pyogenic cocci.

The Blood is affected very considerably by the toxins of the anaerobic bacilli. Thrombosis is common, not only in the primary focus, but also in the smaller vessels throughout the body, the thrombi being of the hyaline, conglutination type. A varying degree of haemolysis seems to occur quite early in the infection, for the spleen often contains a large quantity of haemosiderin, the granules lying free in the pulp, or within macrophages and polynuclear leucocytes. In advanced cases granules of pigment may be found in quantities in the endothelial cells lining capillaries and veins, in the cells of the liver, and in the renal epithelium, especially in the cells of Henle's loops. I have not been able to make any observations on the condition of the cells and haemoglobin content of the circulating blood during life, and an examination of the bone

marrow in a few cases has not revealed any process of regeneration. In some cases the contents of the vessels in the organs examined suggests a slight leucocytosis, but this condition is not constant. The organs are frequently congested, but there is nothing to show that this reaction is at all specific.

The Liver. The liver cells in human organs constantly show cloudy swelling which may be extreme, and occasionally some fatty degeneration. The fat has no distinctive distribution, and neither of these changes can be looked upon as specific.

In experimental animals the hepatic lesions are much more severe. Within twenty-four hours of subcutaneous or intramuscular inoculation the liver cells show extreme changes. There is no fatty degeneration, but the cytoplasm becomes entirely disorganized and appears to undergo partial solution, nothing but an irregular granular débris remaining within an unusually prominent cell wall. The nuclei tend to become hypochromatic but are not otherwise affected. Similar changes occur in animals inoculated intravenously with cultures of bacilli, and in one mouse which lived some forty-eight hours after intravenous inoculation there was, in addition, advanced coarse fatty degeneration.

The Kidney. Here again the changes are constant and severe. The convoluted tubules are chiefly affected. Fatty degeneration never occurs, but the cells undergo advanced cloudy swelling and very often disintegrate. The mitochondria become swollen and irregular in size, and no longer maintain their normal longitudinal arrangement. The cell protoplasm shows an increased affinity for acid dyes, and in some of the experimental animals the appearances almost suggest poisoning with mercury or uranium, so severe are the lesions. There is little change in the nuclei beyond a diminution in the amount of chromatin. It is not uncommon to find coagulum or granular débris in the tubules, and though there is no definite change in the glomeruli, Bowman's capsules sometimes contain a little coagulum. The cells of Henle's loops and the collecting tubules are only slightly affected.

The Spleen. The most constant change in the spleen is due to the altered blood condition, and consists of oedema, occasional thrombosis in the capillary vessels, and pigmentation. Some of the organs have been congested but in others the pulp is empty. An increased number of macrophages has been noted in several specimens, and sometimes these cells are multinucleated.

In two specimens there is a very interesting change in the Malphigian corpuscles. They are considerably hypertrophied and exhibit a central proliferation of endothelial cells, the result being a condition which strongly recalls the germinal node of the lymph gland. The change appears to be analogous to that which occurs in scarlet fever and diphtheria. In some of the nodes the endothelial cells seem healthy, but in others there is a considerable degree of nuclear fragmentation and degeneration of the cytoplasm; and occasionally polynuclear leucocytes can also be detected among the cells. This condition appears to be a reaction to the toxaemia alone, for I have not been able to demonstrate the presence of bacilli.

Lymph glands. These are congested, but do not exhibit any reaction comparable with that seen in the spleen.

Muscle. Slight fatty degeneration sometimes occurs in the heart muscle, but I have found no change in the voluntary muscles apart from the presence of bacteria. In one pigeon I found advanced fatty degeneration of the pectoral muscles into which the toxin of *B. welchii* had been injected some hours previous to death.

The Supra-renal glands. My material was unsuitable for the demonstration of adrenalin, and I have not detected any specific changes in such glands as I have been able to examine.

The Thyroid gland. My material is insufficient to allow of definite conclusions being drawn, but in one case the parenchyma cells show advanced degeneration and desquamation, with an almost complete disappearance of the colloid substance.

B. As the result of the presence of the organism.

Muscle. I have frequently examined portions of the voluntary muscles from different parts of the body in human and experimental cases of gas-gangrene but I have never found in them localized lesions, or toxic changes indicating any specific action of the anaerobic bacilli.

In one case a lesion was present in the heart muscles, the muscle fibres had lost their striation and presented the typical 'ground glass' appearance, and their nuclei no longer stained. Numbers of bacilli were lying free among the desquamated fibres. There was a complete absence of any cellular exudate.

Liver. The liver appears to be a favourable site for the development of lesions. My material contains numerous examples, and I have found typical lesions in the liver of a child who died forty hours after an injury to the arm in which gas-gangrene developed.

The organisms reach the liver by the blood-stream, and can be seen lying free in the sinusoids. I have never seen definite bacterial emboli, nor are the bacilli ingested by the endothelial or parenchyma cells, but isolated organisms appear to be held up mechanically, and eventually small colonies are developed. In a wide zone around these colonies the liver cells are killed. The process appears to be extremely rapid; and of the nature of a coagulation necrosis. At the periphery of the lesion the cells may show cloudy swelling, and the nuclei may be hypochromatic or pycnotic, but for the most part they are peculiarly quiescent. The cells suddenly lose their staining power, but their outlines are retained, and their nuclei can still be distinguished, and should fatty degeneration happen to be present, the fat globules can still be made out. Only in the centre of the lesion do the cells disintegrate. Here they undergo partial solution and become converted into irregular masses of coagulated and semi-digested protein. The bacilli proliferate extensively in the centre of the focus, spreading between the dead cells, but not invading them Sometimes, at the periphery of the lesion the sinusoids may contain a few mono- and polynuclear leucocytes, but there is never any cellular exudate into the lesion. In the late stages gas develops, apparently under pressure, for the cells at the periphery of the vacuoles are compressed. Most of these gas vacuoles are

empty, but occasionally they contain cell débris and bacilli; and bacilli are always found in numbers at their margins.

The bile ducts and supporting tissues of the organs show no change unless they are involved in an unusually large focus when they undergo degeneration in a similar way.

The Kidney. Lesions have been found in the kidney in a few cases. In all essentials they are exactly similar to the hepatic lesions. The presence of colonies of organisms is accompanied by widespread degeneration, and, as might be expected, the changes are first seen in the cells of the convoluted tubules. As in the liver, gas bubbles develop in late stages.

The Spleen. Here the lesions are not uncommon and are of the usual type, though the appearances are complicated by the vascular and toxic changes which have been described. It is noteworthy that even here there is never any cellular exudate, and the bacilli are never phagocytosed.

Lymphatic glands. Infection of the lymphatic glands is probably much more common than is thought. The organisms reach the gland by permeation along the lymph vessels, and give rise to the usual quiet necrosis. Here, again, the lack of any reaction is very striking.

The Supra-renal glands. In one case I found a lesion which conformed in every respect with the lesions in other organs.

Nervous System. The peripheral nerves in the local lesions show changes similar to those in the muscles and other tissues. I have not found lesions in the central nervous system, but in one case there was a condition of cerebral meningitis, apparently due to one of the anaerobic bacilli. The lesion, in this case, had the character of an ordinary acute meningitis. The membrane was congested and covered with a polynuclear leucocytic exudate, and there was active phagocytosis of the bacilli by the leucocytes. Many bacilli were present in the vessels of the underlying cerebral cortex, but the nerve cells showed no change.

DESCRIPTION OF PLATES

1. *B. welchii.* Film preparation, 18-hour-old culture on alkaline egg. (Henry, Fig. 36).
2. *B. welchii.* Film preparation, 3 days' growth on alkaline meat, showing short coccoid forms. (Henry, Fig. 37.)
3. *B. welchii.* Film preparation from infected haemothorax fluid showing streptobacillary form. (Henry, Fig. 38A.)
4. *B. welchii.* Filamentous and chain forms grown on alkaline egg agar for 24 hours. (Henry, Fig. 38B.)
5. *B. welchii.* 24-hour culture on alkaline egg agar to show involution forms. (Henry, Fig. 39.)
6. *B. welchii.* 2 days' growth on human serum to show spore formation. (Henry, Fig. 40.)
7. *B. welchii.* 24-hour culture in starch broth to show spore formation. (Original.)
8. *Vibrion septique.* 24–48 hour meat culture. ×1500. (Robertson, *B. M. J.*, Fig. 1.)
9. *Vibrion septique.* Noguchi tube culture showing rods, spores, and a 'citron' type. ×1500.
10. *Vibrion septique.* Post-mortem specimen from muscle of guinea-pig; citrons and club-shaped types are shown. ×1500.
11. *Vibrion septique.* From liver of guinea-pig showing 'citron', 'bulb' and oval forms. ×1500.
12. *Vibrion septique.* From liver of guinea-pig showing long filaments, &c. ×1000.
13. *Vibrion septique.* 'Citrons' from haemorrhagic blister fluid of patient suffering from gas-gangrene. ×1500.
14. *Vibrion septique.* 48 hours' growth on serum agar. ×1000. (McIntosh, Plate I, Fig. 4).
15. *Vibrion septique.* Noguchi culture. ×1000. (McIntosh, Plate I, Fig. 5.)
16. *Vibrion septique.* Smear from peritoneum of a mouse. × 500. (McIntosh, Plate I, Fig. 6.)
17, 18. *Vibrion septique.* Broth cultures. × 1000. (McIntosh, Plate I, Figs. 7 and 8.)
19. *B. oedematiens.* Egg broth culture. (Henry, Fig. 45A.)
20. *B. oedematiens.* From surface colony. (Henry, Fig. 45B.)
21. *B. chauvoei.* Four days' broth culture. ×1000. (McIntosh, Plate II, Fig. 1.)
22. *B. chauvoei.* 24-hour Noguchi tube. ×1000. (McIntosh, Plate II, Fig. 2.)
23. *B. chauvoei.* Smear from muscle of guinea-pig. ×1000. (McIntosh, Plate II, Fig. 3.)
24. *B. histolyticus.* 16 hours' growth in alkaline meat. ×1500. (Original).
25, 26. *B. botulinus.* Broth culture. ×1000. (McIntosh, Plate III, Figs. 4 and 5.)
27. *B. sporogeñes.* Noguchi tube culture, 3 days' growth. × 1000. (McIntosh, Plate IV, Fig. 4.)

27A. *B. sporogenes.* 48 hours' agar. (McIntosh, Plate II, Fig. 4).

28. *B. tertius.* 5-day-old colony on alkaline egg agar. ×1300. (Henry, Fig. 42.)
29. *B. cochlearius.* Broth culture. ×1000. (McIntosh, Plate II, Fig. 9.)
30. *B. tetanomorphus.* 48 hours' growth on agar. ×2000. (McIntosh, Plate II, Fig. 4.)
31. *B. tertius.* (Original.)
32. *B. cochlearius.* (Original.)
33, 34. *B. tetanomorphus.* 48 hours' growth on agar. (Original.)
35. *B. fallax.* Broth culture two days old. × 1300. (Henry, Fig. 43.)
36. *B. aerofetidus.* 3 days' culture in alkaline egg broth. × 1300. (Henry, Fig. 44.)
37. *B. bifermentans.* 48 hours' growth meat medium. ×1500. (Original.)
38. *B. sphenoides.* 48 hours' growth starch broth. (Original.)

39. *B. sphenoides.* 48 hours' growth potato broth. (Original.)
40. *B. butyricus.* Broth culture. ×1000. (McIntosh, Plate II, Fig. 8.)
41. *B. welchii.* 24-hour colonies on alkaline egg agar. ×6. (Henry, Fig. 22.)
42. *B. welchii.* 48-hour growth colony in agar. (McIntosh, Plate V, Fig. 1.)
43. *B. oedematiens.* 3 days old colonies on egg agar. ×6. (Henry, Fig. 34.)
44. *B. histolyticus.* 3 days old colonies on egg agar. ×6. (Henry, Fig. 35.)
45. *Vibrion septique.* 24 hours' culture. (McIntosh, Plate VI, Fig. 3.)
46. *Vibrion septique.* 24 hours' surface colony. (McIntosh, Plate VII, Fig. 2.)
47. *B. sporogenes.* Surface colonies 3 days old. × 6. (Henry, Fig. 28.)
48. *B. sporogenes.* Surface colonies: egg agar. Slow-growing colonies 2 days old. ×6. (Henry, Fig. 27.)
49. *B. tertius.* Surface colonies on egg agar. ×6. (Henry, Fig. 29.)
50. *B. fallax.* 3 days old colonies: egg agar. ×6. (Henry, Fig. 30.)
51. *B. aerofetidus.* 3 days old colonies: egg agar. ×6. (Henry, Fig. 33.)
52. *B. welchii.* 24 hours' growth on serum agar slope. (McIntosh, Plate XII, Fig. 5.)
53. *B. sporogenes.* Growth on serum agar slope 1 week. (McIntosh, Plate XII, Fig. 3.)
54. *B. tertius.* 48-hour growth on serum agar slope. (McIntosh, Plate XIII, Fig. 5.)
55. *Vibrion septique.* 4 days' growth on serum agar slope. (McIntosh, Plate XIII, Fig. 1.)
56. *B. welchii* (Strain 82). Milk culture, 24 hours old, to show stormy fermentation. Note the disappearance of the cream layer, the disruption of the casein clot, and the displacement of shreds of clot upwards through the paraffin layer.
57. *B. welchii* (Strain Pasteur). Alkaline meat culture, 1 week old, and showing reddening of the meat. Contrast this with the normal meat in Plate XXVII, Fig. 20.
58. *B. welchii* (Strain 82). Culture in alkaline egg, 5 days old. Note the diffuse opacity due to fine friable clot. There is no shrinkage and no sign of digestion. Contrast this with normal alkaline egg in Plate XXVII, Fig. 21.
59. *B. sporogenes* (Strain Pasteur). Milk culture, 1 month old. To show undisturbed cream layer and complete digestion of the milk.
60. *B. sporogenes* (Strain Pasteur). Culture in alkaline milk, 1 week old. To show digestion and blackening.
61. *B. sporogenes* (Strain Harwood). Culture in alkaline meat, 1 week old. To show marked blackening of the upper layers of meat.
62. *B. sporogenes* (Strain Pasteur). Culture in alkaline egg. To show cylindrical coagulum which has undergone gradual shrinkage and partial digestion.
63. *B. tertius* (Strain 68). Milk culture, 6 weeks old. To show solid clot fissured by gas bubbles.
64. *B. tertius* (Strain Benson). Culture in alkaline meat, 14 days old. To show pinking of meat and commencing bleaching on the surface.
65. *B. fallax* (Strain Trucol). Milk culture, 1 month old, showing strong solid clot disrupted by bubbles, and with orange colour at the surface.
66. *B. fallax* (Strain 92). Milk culture, 5 days old, showing separation into a turbid whey and a soft friable clot which is slightly disrupted by gas. There is no dislocation of the cream layer. Bubbles have collected on the air surface of the paraffin layer.
67. *B. aerofetidus* (Weinberg). Culture in milk, 36 hours old, and showing disruption of casein clot by gas. The fermentation is not so vigorous as, and develops later than, the reaction given by *B. welchii.*
68. *B. aerofetidus* (Weinberg). Growth in alkaline meat, 7 days old, showing reddening of the meat and slight blackening of the upper layers.
69. *B. oedematiens* (Strain Domange). Growth in milk, after 4 months' incubation, to show slight separation of whey from subjacent casein flocculi.
70. *B. oedematiens* (Strain Domange). Culture in alkaline meat, after 15 days, to show pinking of meat and extensive bleaching from surface downwards.
71. *B. histolyticus* (Weinberg). Milk culture, 2 days old, showing complete digestion, with no disturbance of the cream layer.
72. *B. histolyticus* (Weinberg). Culture in alkaline meat, 4 days old, to show digestion and formation of glistening white balls of tyrosin.

73. Ordinary milk under paraffin.
74. Alkaline meat medium under paraffin.
75. Alkaline egg medium under paraffin.
76. Lesions produced in a guinea-pig by the injection of a culture of *Vibrion septique* into the muscles of the thigh. The subcutaneous tissues over the abdomen and thorax are the seat of a clear, deep red, sero-sanguinolent oedema in which there are extensive collections of gas bubbles. The abdominal muscles and inguinal fat are intensely congested.
77. Lesions produced in a guinea-pig by the injection of a culture of *Bacillus welchii* into the muscles of the thigh. In comparison with those of *V. septique* the oedema is only slightly blood-stained, the formation of gas is usually less marked, and the muscles themselves are pale and looked bleached.
78. Lesions produced in a guinea-pig by the injection of a culture of *Bacillus oedematiens* into the muscles of the thigh. The characteristic feature is a clear colourless gelatinous oedema, while the abdominal muscles are for the most part unaltered. In general, gas formation if present at all is trivial.

(Figs. 56–75 from Henry (Figs. 1–9 and 11–21).)

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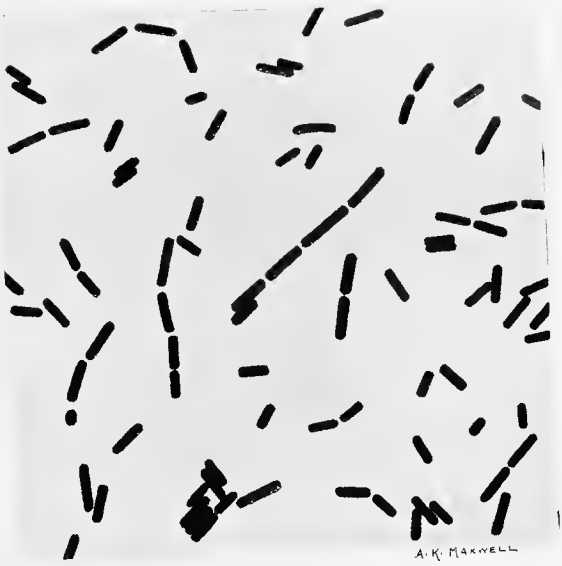

FIG. 1

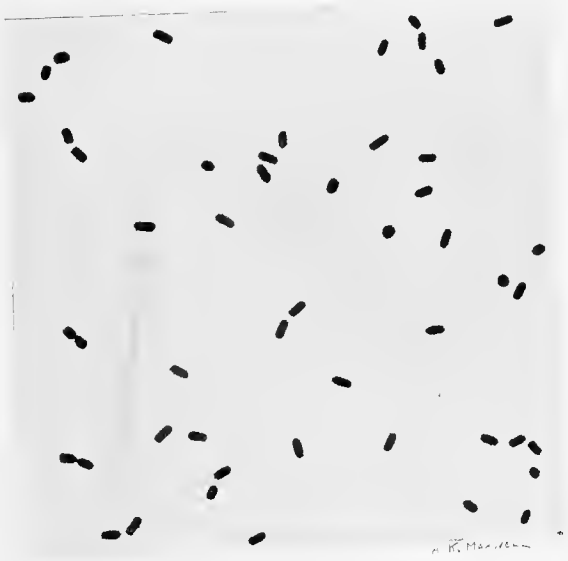

FIG. 2

FIG. 3

FIG. 4

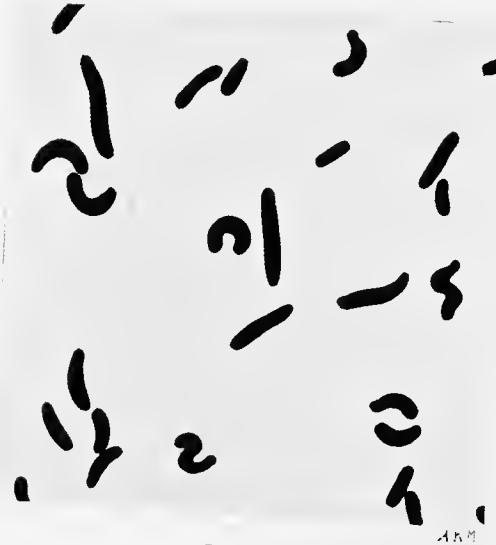

FIG. 5

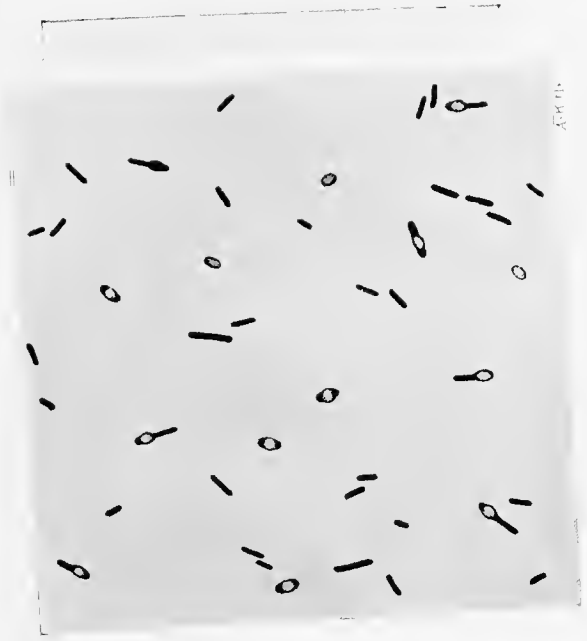

FIG. 6

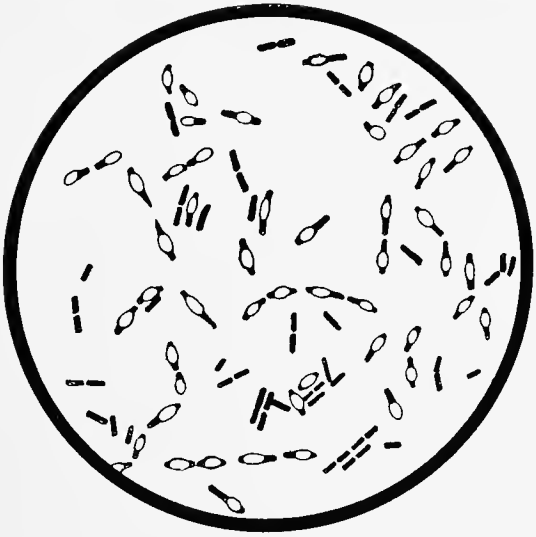

FIG. 7

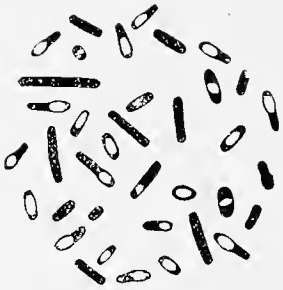

FIG. 8

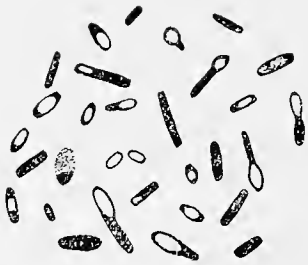

FIG. 9

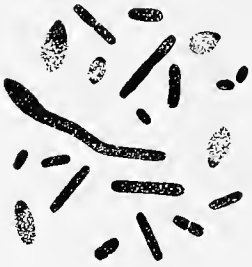

FIG. 10

FIG. 11

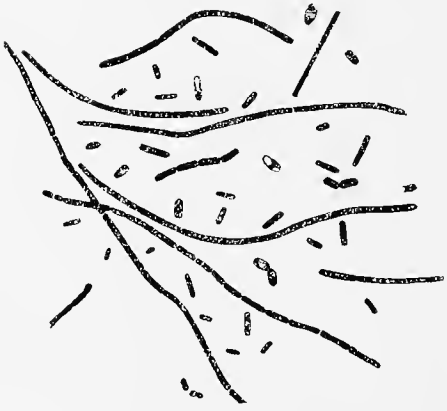

FIG. 12

FIG. 13

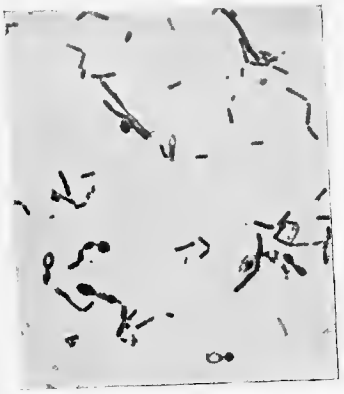

FIG. 14

FIG. 15

FIG. 16

FIG. 17

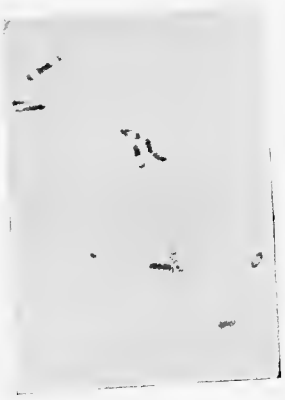

FIG. 18

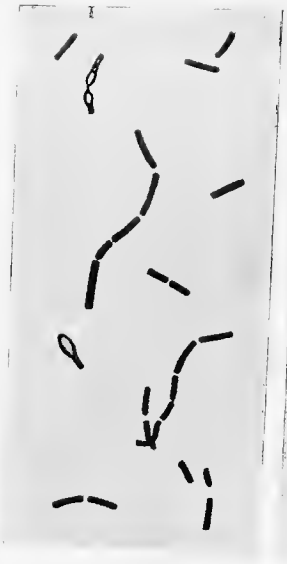

FIG. 19

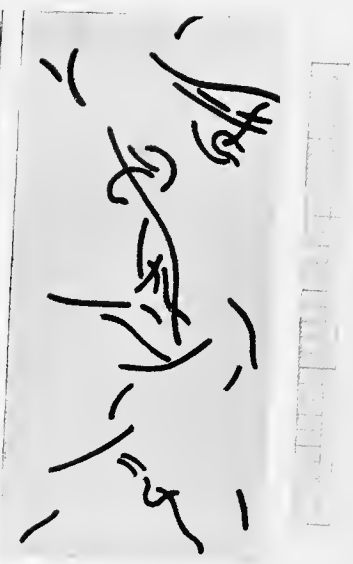

FIG. 20

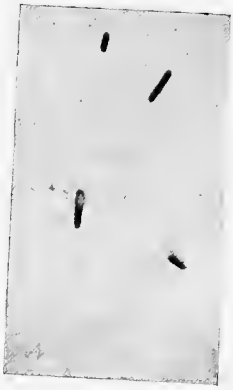

FIG. 21

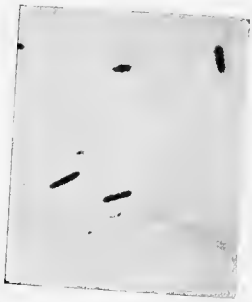

FIG. 22

FIG. 23

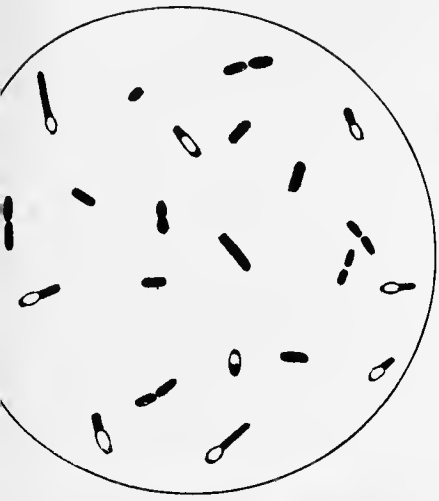

FIG. 24.

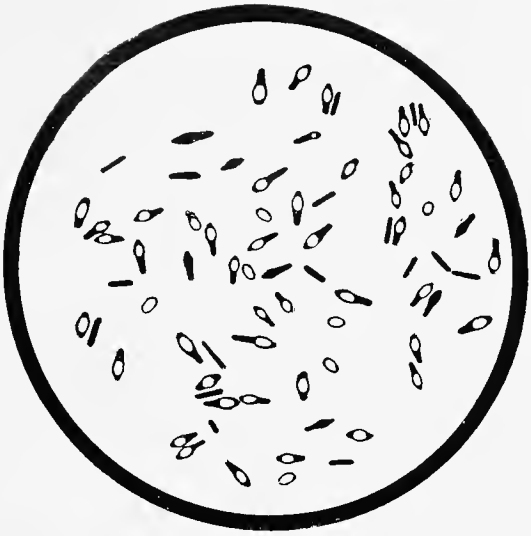

FIG. 27 A

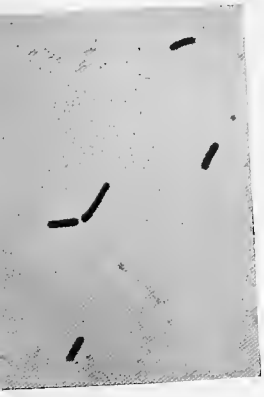

FIG. 25

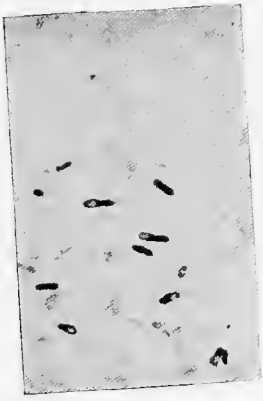

FIG. 26

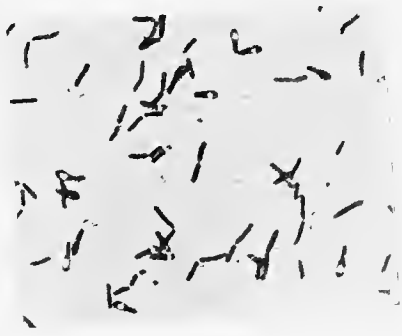

FIG. 27

FIG. 28

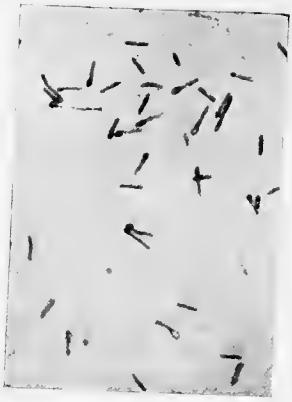

FIG. 29

FIG. 30

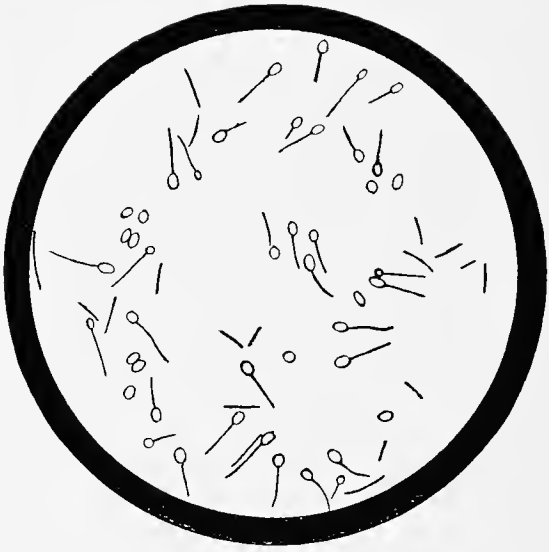

FIG. 31

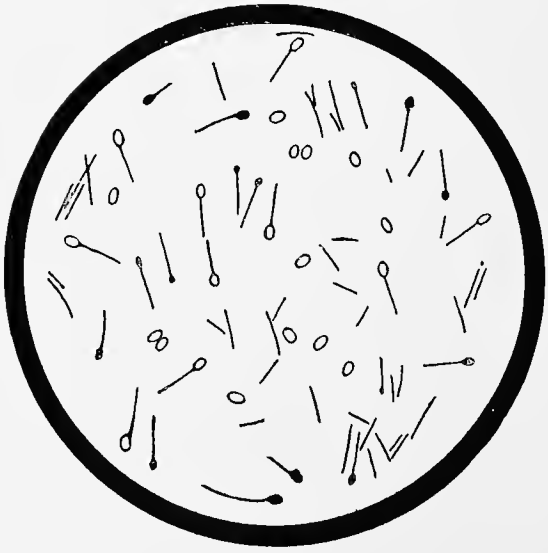

FIG. 32

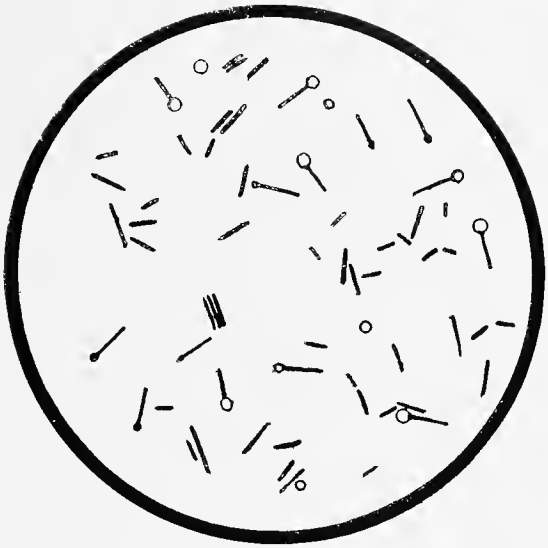

FIG. 33

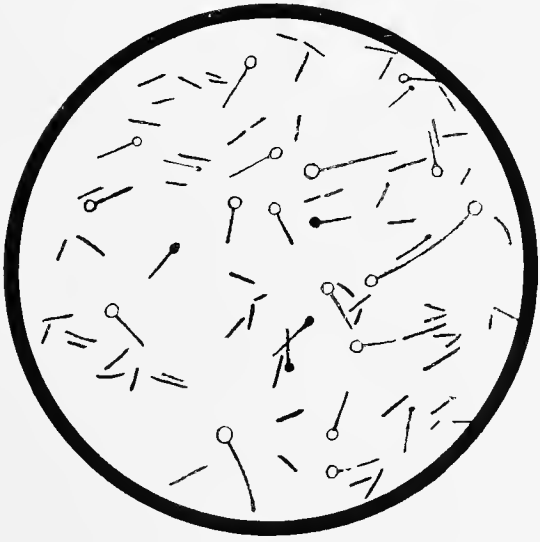

FIG. 34

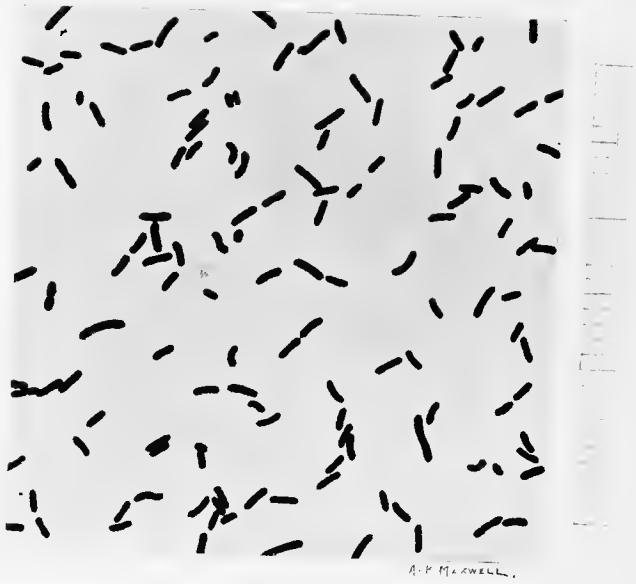

FIG. 35

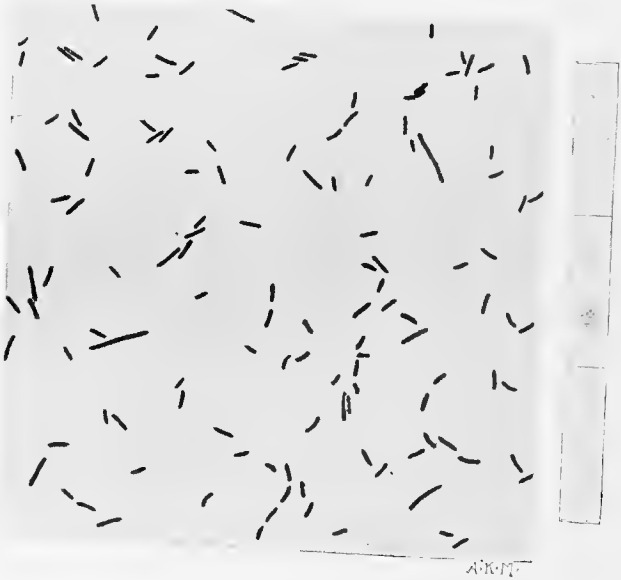

FIG. 36

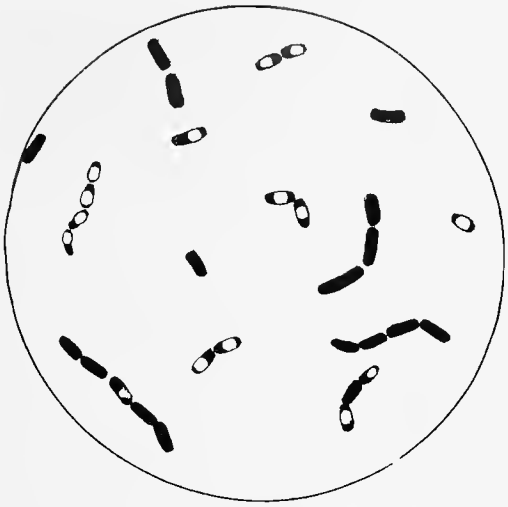

FIG. 37

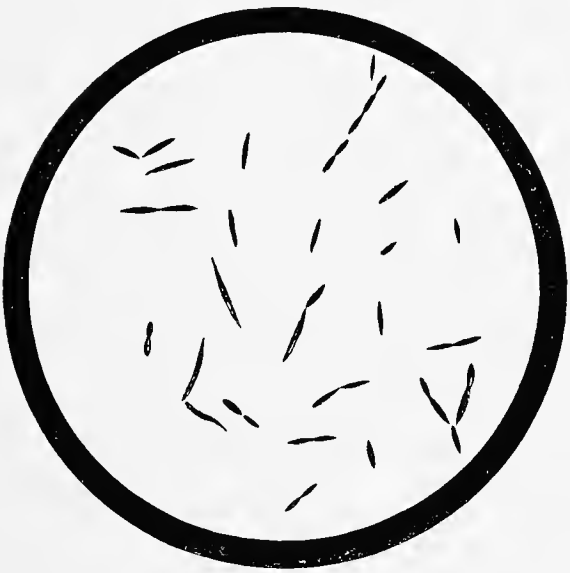

FIG. 38

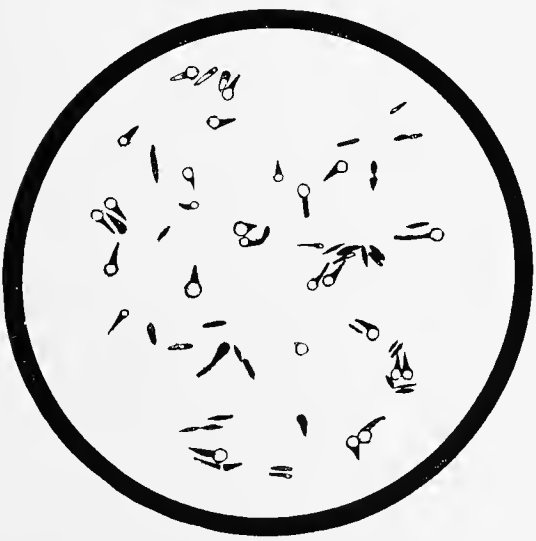

FIG. 39

FIG. 40

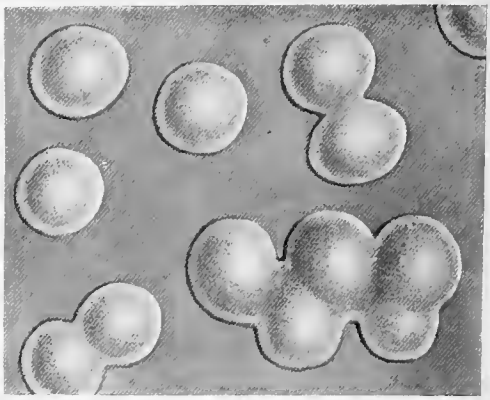

FIG. 41

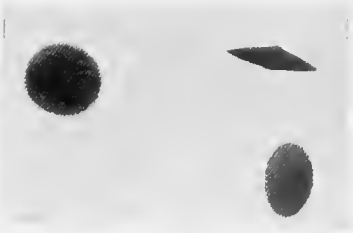

FIG. 42

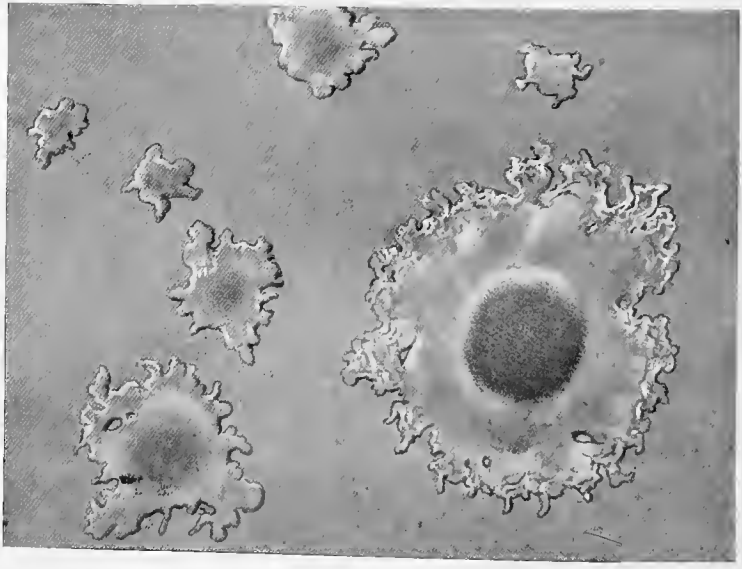

FIG. 43.

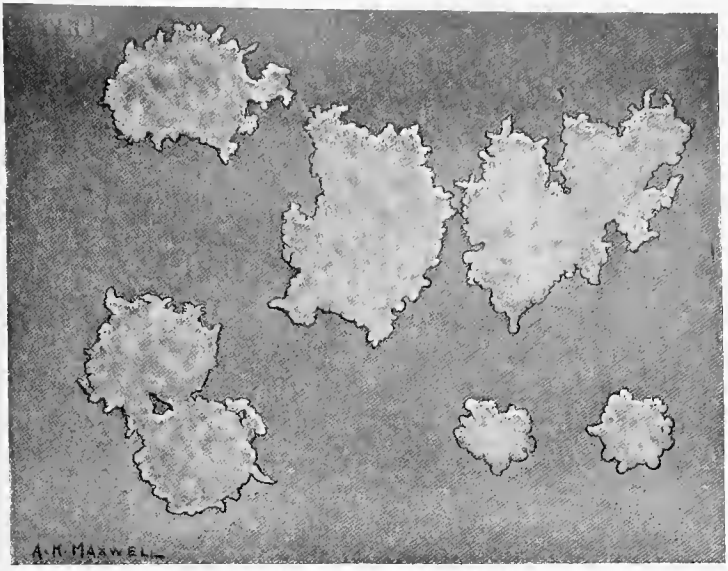

FIG. 44

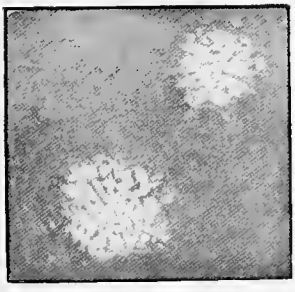

FIG. 45

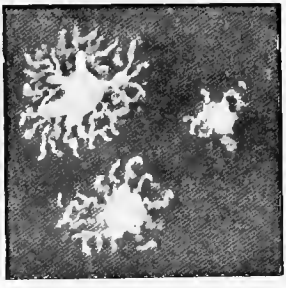

FIG. 46

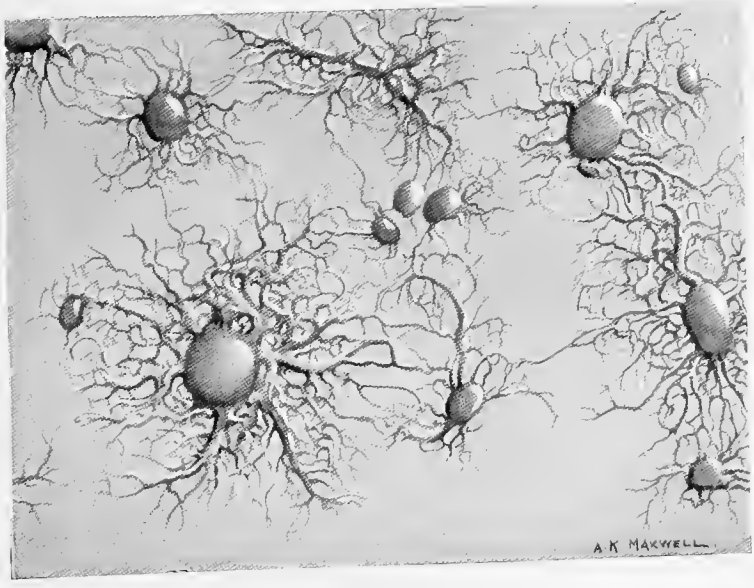

FIG. 47

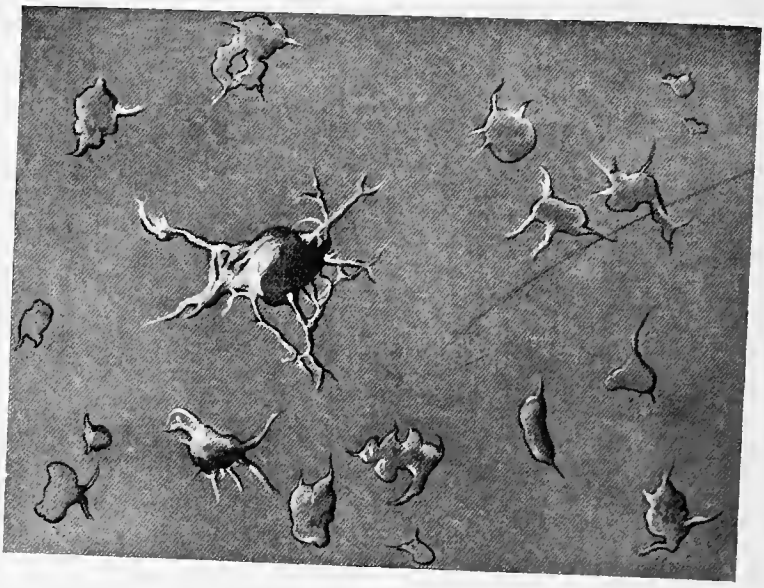

FIG. 48

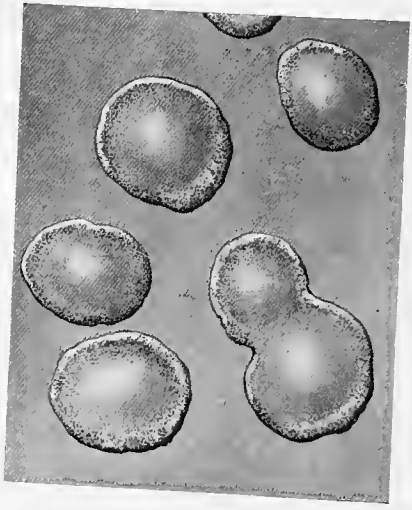

FIG. 49

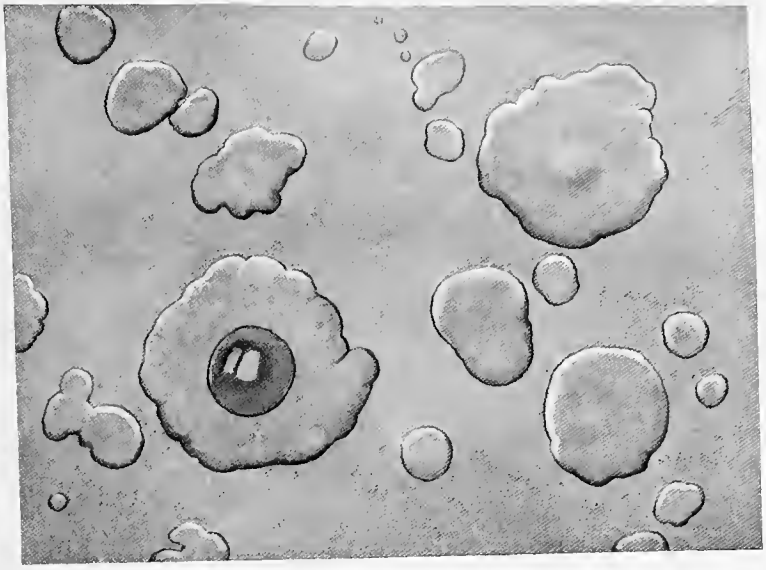

FIG. 50

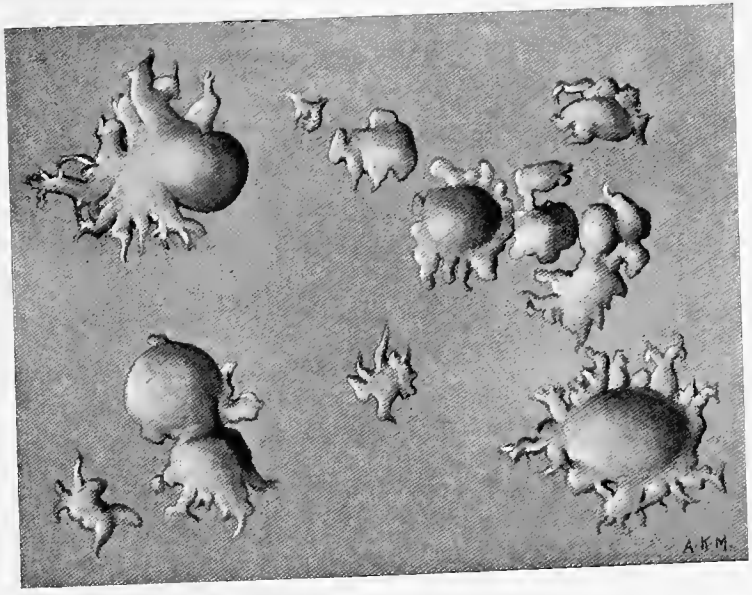

FIG. 51

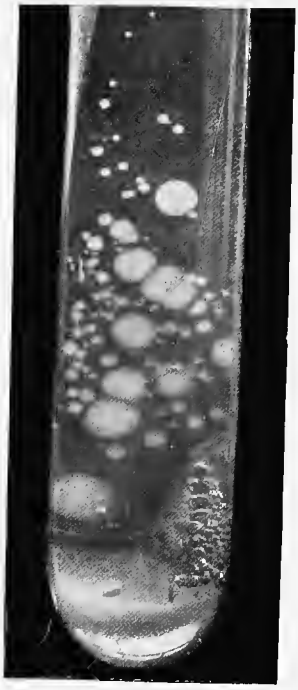

FIG. 52

FIG. 53

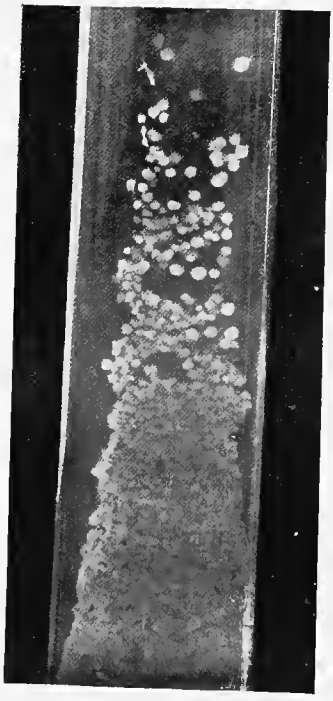

FIG. 54

Fig. 56.

Fig. 57.

Fig. 58.

Fig. 59.

Fig. 60.

Fig. 61.

Fig. 62.

Fig. 63.

Fig. 64.

Fig. 65.

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Fig. 68

Fig. 69.

Fig. 70.

Fig. 71.

Fig. 72.

Fig. 73.

Fig. 74.

Fig. 75.

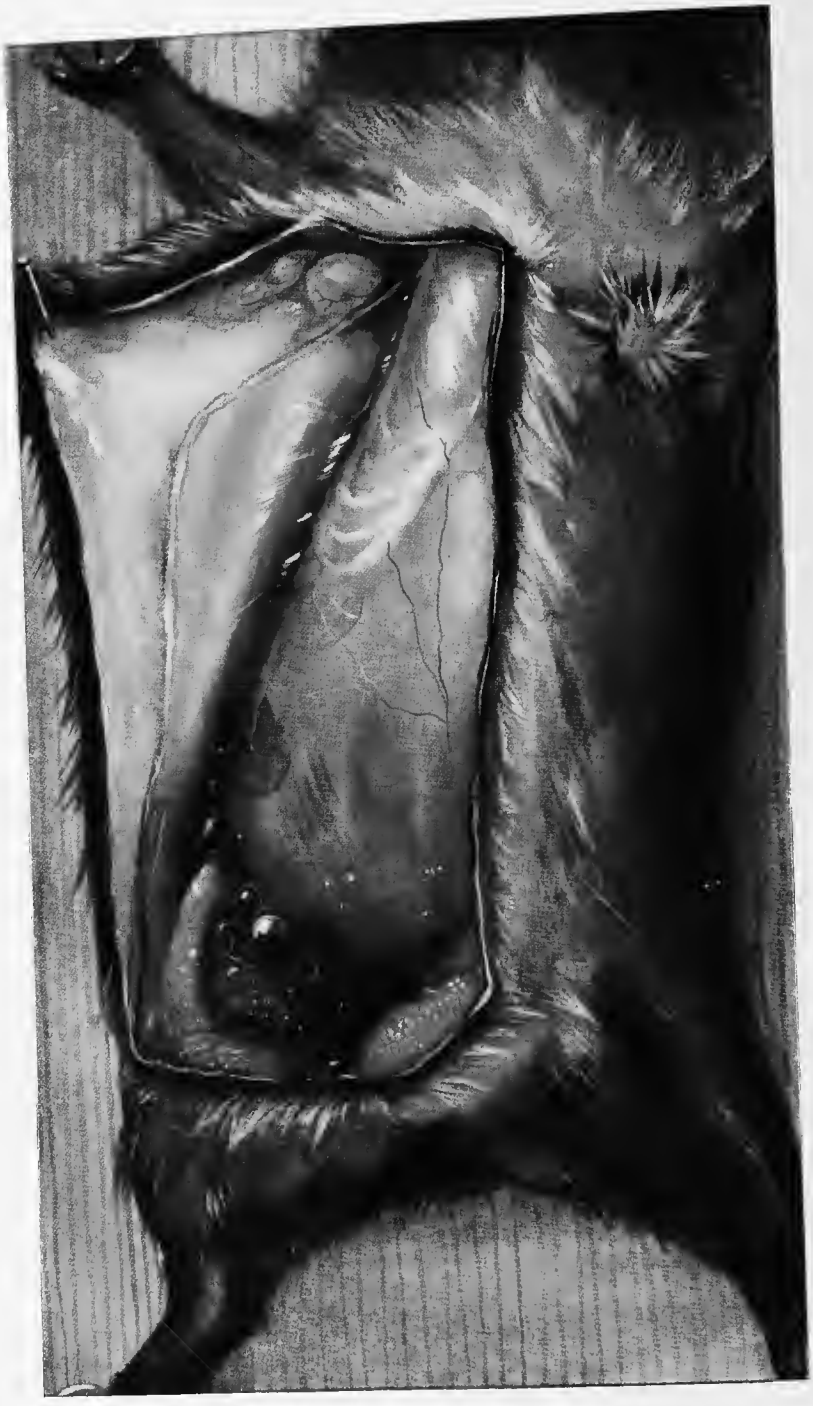

Figure 76

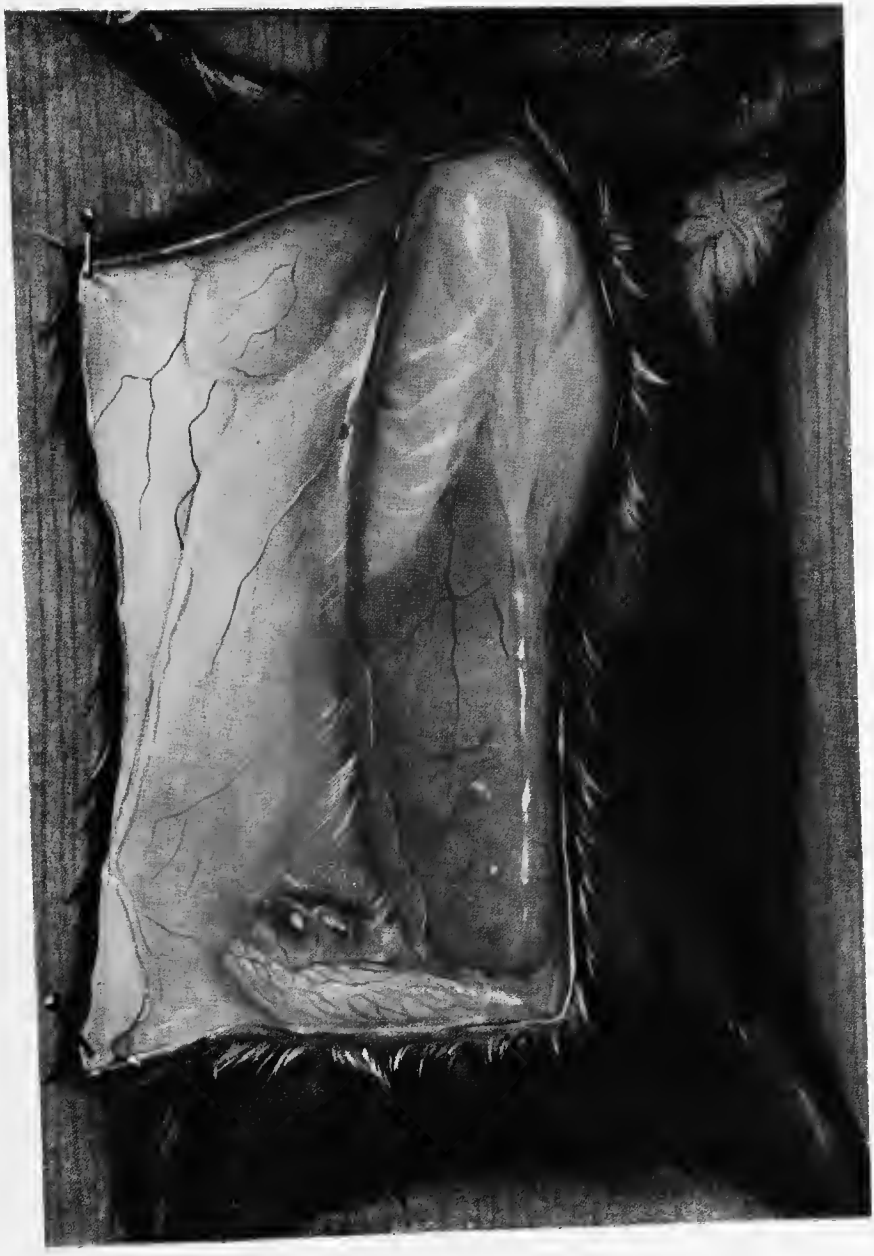

Figure 77

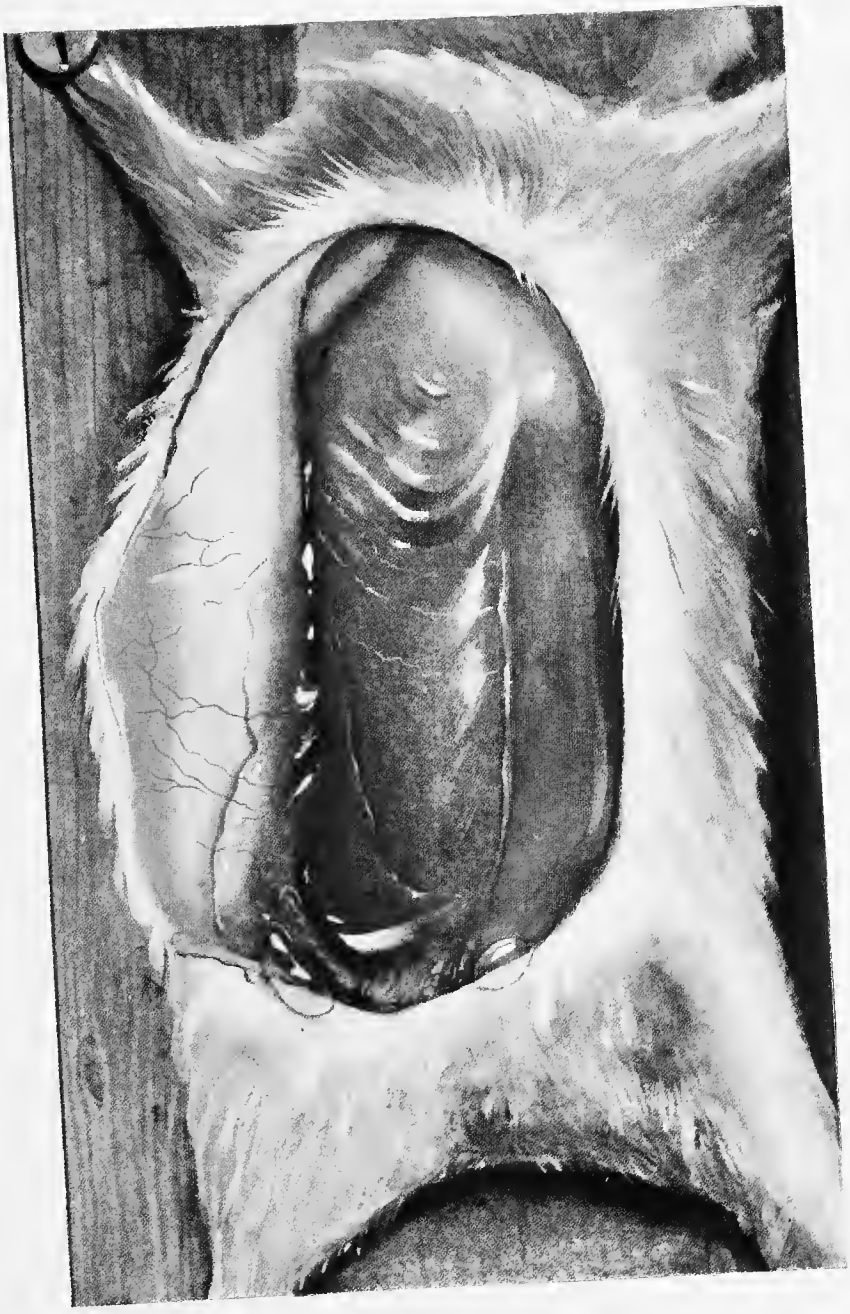

Figure 78

Hospital, Haslar, 1915–16.

IV. Report upon combined Clinical and Bacteriological Studies of Dysentery Cases from the Mediterranean. Price 4*s.* 6*d.*, post free 4*s.* 8*d.*

No. 7. V. Report upon 2,360 Enteritis 'Convalescents' received at Liverpool from various Expeditionary Forces. Price 6*s.*, post free 6*s.* 2½*d.*

No. 15. A Study of 1,300 Convalescent Cases of Dysentery from Home Hospitals: with special reference to the Incidence and Treatment of Amoebic Dysentery Carriers. Price 1*s.* 3*d.*, post free 1*s.* 4*d.*

No. 29. A Contribution to the Study of Chronicity in Dysentery Carriers. Price 9*d.*, post free 10½*d.*

No. 30. An Investigation of the Flexner-Y group of Dysentery Bacilli. Price 1*s.*, post free 1*s.* 1½*d.*

No. 40. Studies of Bacillary Dysentery occurring in the British Forces in Macedonia. Price 3*s.*, post free 3*s.* 2*d.*

No. 42. A Study of the Serological Races of the Flexner Group of Dysentery Bacilli. Price 2*s.*, post free 2*s.* 2*d.*

Venereal Diseases:

No. 23. An Analysis of the Results of Wassermann Reactions in 1,435 Cases of Syphilis or Suspected Syphilis. Price 2*s.*, post free 2*s.* 1½*d.*

No. 41. I. An Investigation into the Ultimate Results of the Treatment of Syphilis with Arsenical Compounds.

II. A Clinical Study of the Toxic Reactions which follow the Intravenous Administration of '914'. Price 2*s.*, post free 2*s.* 1½*d.*

No. 44. Reports of the Special Committee upon the Manufacture, Biological Testing, and Clinical Administration of Salvarsan and of its Substitutes. No. I. Price 1*s.*, post free 1*s.* 1½*d.*

No. 45. Unsuspected Involvement of the Central Nervous System in Syphilis. Price 1*s.*, post free 1*s.* 1½*d.*

No. 47. The Accuracy of Wassermann Tests, applied before and after death, estimated by Necropsies. I. The Wassermann Test applied before death. Price 2*s.* 6*d.*, post free 2*s.* 8*d.*

No. 55. I. Results of the Examination of Tissues from Eight Cases of Death following Injections of Salvarsan.

II. The Influence of Salvarsan Treatment on the Development and Persistence of Immunity, as indicated by Measurements of Agglutinins. Price 3*s.*

(Vide infra, *Reports of the Committee upon Pathological Methods*, Nos. I–IV.)

Alcohol:

No. 31. Alcohol—Its Absorption into and Disappearance from the Blood under different conditions. Price 1*s.*, post free 1*s.* 1½*d.*

No. 34. The Influence of Alcohol on Manual Work and Neuro-muscular Co-ordination. Price 2*s.*, post free 2*s.* 1½*d.*

No. 56. The Effects of Alcohol and some other Drugs during Normal and Fatigued Conditions. Price 1*s.*, post free 1*s.* 1½*d.*

Reports of the Committee upon Pathological Methods:

No. 14. I. The Wassermann Test. Price 3*d.*, post free 4½*d.*

No. 19. II. The Laboratory Diagnosis of Gonococcal Infections.

III. Methods for the Detection of Spirochaetes. Price 1*s.*, post free 1*s.* 1½*d.*

No. 21. IV. The Diagnostic Value of the Wassermann Test. Price 1*s.*, post free 1*s.* 1½*d.*

No. 35. V. The Reaction of Media. Price 6*d.*, post free 7½*d.*

No. 51. VI. The Laboratory Diagnosis of Acute Intestinal Infections, including the Principles and Practice of the Agglutination Tests. Price 4*s.* 6*d.*, post free 4*s.* 8½*d.*

Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions:

No. 25. I.–VII. Wound-Shock and Haemorrhage. Price 4*s.*, post free 4*s.* 6*d.*

eports of the Air Medical Investigation Committee:

No. 37. VIII. The Effects of Diminished Tension of Oxygen, with especial reference to the Activity of the Adrenal Glands.

IX. The Ear in relation to certain Disabilities in Flying. Price 1*s.*, post free 1*s.* 1½*d.*

No. 53. The Medical Problems of Flying (including Reports Nos. I–VII). Price 6*s.*, post free 6*s.* 4½*d.*

eports of the Committee upon Accessory Food Factors (Vitamines):

No. 38. Report on the Present State of Knowledge concerning Accessory Food Factors (Vitamines). Price 4*s.*, post free 4*s.* 3*d.*

eports of the Committee upon Anaerobic Bacteria and Infections:

No. 39. Report on the Anaerobic Infections of Wounds and the Bacteriological and Serological Problems arising therefrom. Price 6*s.*, post free 6*s.* 4*d.*

No. 8. Report upon Soldiers returned as Cases of 'Disordered Action of the Heart' (D.A.H.), or 'Valvular Disease of the Heart' (V.D.H.). Price 1*s.*, post free 1*s.* 1½*d.*

No. 9. A Report upon the use of Atropine as a Diagnostic Agent in Typhoid Infections. Price 1*s.*, post free 1*s.* 1½*d.*

No. 10. The Mortalities of Birth, Infancy, and Childhood. Price 1*s.* 6*d.*, post free 1*s.* 8*d.*

No. 11. The Causation and Prevention of Tri-nitro-toluene (T.N.T.) Poisoning. Price 1*s.*, post free 1*s.* 2*d.*

No. 12. The Classification and Study of the Anaerobic Bacteria of War Wounds. Price 2*s.*, post free 2*s.* 2½*d.*

No. 13. An Enquiry into the Composition of Dietaries, with special reference to the Dietaries of Munition Workers. Price 9*d.*, post free 10½*d.*

No. 16. A Report on the Causes of Wastage of Labour in Munition Factories. Price 1*s.* 6*d.*, post free 1*s.* 7½*d.*

No. 20. A Study of Social and Economic Factors in the Causation of Rickets, with an Introductory Historical Survey. Price 2*s.*, post free 2*s.* 2*d.*

No. 24. A Report on the Investigation of an Epidemic caused by Bacillus aertrycke. Price 9*d.*, post free 10½*d.*

No. 32. The Science of Ventilation and Open Air Treatment. Part I. Price 10*s.*, post free 10*s.* 6*d.*

No. 36. Studies of Influenza in the Hospitals of the British Armies in France, 1918. Price 3*s.* 6*d.*, post free 3*s.* 8½*d.*

No. 43. Albuminuria and War Nephritis among British Troops in France. Price 3*s.* 6*d.*, post free 3*s.* 8½*d.*

No. 48. A Report on the probable Proportion of Enteric Infections among Undiagnosed Febrile Cases invalided from the Western Front since October, 1916. Price 3*s.*, post free 3*s.* 2*d.*

No. 49. On the Destruction of Bacteria in Milk by Electricity. Price 9*d.*, post free 10½*d.*

No. 52. The Science of Ventilation and Open Air Treatment. Part II. Price 6*s.*, post free 6*s.* 5*d.*

No. 57. Studies in Wound Infections.

The following were published under the direction of the Medical Research Committee:

Milk and its Hygienic Relations, by Janet E. Lane-Claypon, M.D., D.Sc. (Lond.). Price 9*s.* net. [Longmans, Green & Co.]

The Amoebae living in Man, by Clifford Dobell, F.R.S. Price 7*s.* 6*d.* net. [Bale, Sons & Danielsson, Ltd.]

In addition to the publications contained in the list given above, numerous memoirs upon work aided by the Medical Research Council have appeared in Scientific Journals.