THE BACTERIA OF GANGRENOUS WOUNDS.

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The cases which provided the material for this investigation were selected from those undergoing treatment at the 3rd Northern General Hospital, Sheffield. The injuries were of a severe nature, and in nearly all there was evidence of the presence of necrotic or gangrenous tissue in the wound. Comparatively few, however, presented the clinical features of emphysematous gangrene.

Methods Employed.

The material used for examination consisted of fragments of necrotic tissue or of the discharges from the deeper parts of the wound.

The procedure usually adopted was as follows: Films were prepared and stained by various methods. Attention was directed to the cells and to the appearance and numbers of bacteria present in the discharge.

Methods for the Isolation of Aerobic Organisms.

Plate cultures were made on blood agar, and in some cases on MacConkey's medium. The resulting colonies were identified by the usual methods.

Methods for the Isolation and Identification of Anaerobic Organisms.

A tube containing about 5 c.cm. of broth was heavily inoculated with the material to be examined. In some cases a small piece of necrotic tissue was placed bodily into the tube, in others the material present on one or two swabs was thoroughly emulsified in the broth. In place of broth the following medium was employed on several occasions.

"Egg Broth."

The white and yolk of one egg were thoroughly beaten up by vigorous shaking in a stout glass bottle containing a liberal supply of glass beads; 300 c.cm. of distilled water were then added, and the mixture was thoroughly shaken. The bottle containing the mixture was then placed in a water bath which was slowly brought to the boil. This temperature was maintained for half an hour. During the whole period of heating the bottle was constantly and violently shaken. The result was a loose porridge-like mass consisting of finely divided particles of egg suspended in fluid. The medium was distributed in quantities of 50 to 100 c.cm. in wide-mouthed bottles, which were plugged and sterilized in the autoclave.

"Egg broth" prepared in this way proved to be very useful as a culture medium for the growth of anaerobic micro-organisms. It is particularly useful for the growth of *B. oedematis maligni*.

This egg broth or ordinary nutrient broth, after liberal inoculation, was heated for half an hour at a temperature of 80° C. It was then incubated in a Bulloch's chamber at 37° C. for periods which varied from two to twenty days. After this preliminary incubation (1) films were prepared, stained, and examined; (2) guinea-pigs were inoculated subcutaneously with one or two cubic centimetres of the broth; (3) agar, blood agar, or glucose agar plates were prepared, inoculated from the broth, placed in a Bulloch's chamber, and incubated for a period of three to seven days.

It was found that glucose agar was the most suitable medium for these anaerobic plate cultures. Subcultures from the resulting colonies were made on Dorset's egg medium, which proved admirably adapted for the needs of *B. aerogenes capsulatus* and *B. oedematis maligni*. It is probably better to subculture tetanus on glucose agar. The cultural characteristics of the various strains of anaerobic bacteria were examined on various culture media.

A more detailed account of this investigation is to be published in the *Journal of the Royal Army Medical Corps*.

General Table.

No. of Case.	Gas observed in Tissues.	Symptoms of Tetanus.	Fatal.	<i>B. oedematis maligni</i> .	<i>B. aerogenes capsulatus</i> .	<i>B. tetani</i> .
1	0	+	0	+	+	0
2	+	0	+	+		0
3	0	0	0			0
4	0	0	0	0	0	0
5	0	0	0		0	0
6	0	+	0	+	0	+
7	0	0	0	+	0	0
8	0	0	0	+		0
9	0	0	0	+		0
10	0	0	0	+		+
11	0	0	0	+	+	0
12	0	0	0	+	+	0
13	0	0	0	0	+	0
14	+	0	0	+	+	+
15	0	+	0		+	0
16	0	+	+	0		
17	+	0	+		0	0
18	+	0	0		+	0

Comment on Results.

The eighteen cases enumerated in Table I came under observation during a period of ten months from September, 1914, to June, 1915. The patients had been wounded in various parts of France and Belgium during this period. Although the number of cases is small, the series comprises patients wounded in widely separated districts and under very different climatic conditions, and in the circumstances the absence of variety in the bacteriological findings is somewhat noteworthy. The cases investigated were those in which there had been gross destruction of tissue, with subsequent necrosis and gangrene. The discharge from the wound was, in the majority of cases, excessively offensive.

The aerobic bacteria which were isolated call for little comment. Streptococci were isolated from nearly every case. No attempt was made to define accurately the variety of streptococcus, but nearly all those isolated appeared to belong to one type, which in films appeared as a small diplococcus, or more rarely as a chain of four or six members. It grew freely on blood-smeared agar and on ordinary agar in small translucent colonies. Films made from blood agar cultures showed short chains. Staphylococci were isolated less frequently than might have been anticipated. When present, they were not as a rule numerous.

B. coli was only isolated from four cases. The only other aerobic organism of any interest was a *proteus* bacillus from case No. 5.

The Anaerobic Bacteria.

Only three varieties of anaerobic bacteria were isolated—the *B. aerogenes capsulatus*, *B. oedematis maligni*, and the *B. tetani*.

These varieties belong to one group of bacteria, whose normal habitat is usually stated to be highly cultivated earth and dung; their constant presence in wounds of the type investigated was to be anticipated.

Morphology and Staining Reactions.

B. aerogenes capsulatus and *B. oedematis maligni* differ enormously both in shape and in staining reactions under different cultural conditions, and the identification of either from the appearances seen in a film preparation alone may often be difficult. The results obtained by Gram's method of staining differ greatly with the nature of the culture medium and the age of the culture. The influence of the variety of culture medium employed on the formation of spores is also well marked.

The relative rate of growth of the different varieties is a matter of some interest from the practical standpoint, and

is a factor which must be allowed for in any attempt to isolate bacteria of this group in pure culture. In a mixed culture made by inoculating a tube of broth with the discharge obtained from a wound the different varieties appear after different periods of time, as it were in crops. After two or three days' incubation the broth may appear to contain a pure culture of *B. aerogenes capsulatus*. After a further period of incubation the *B. oedematis maligni* is often the predominant organism. Tetanus bacilli seem to require a still longer interval for multiplication, and may appear first after ten days' incubation. A result of this kind was obtained on several occasions, and in the later cases of the series the original broth culture was subjected to a routine examination at various intervals after inoculation. The preparation of pure cultures was thereby considerably facilitated. It is possible that a similar method might prove useful in dealing with other groups of bacteria.

The cultural characteristics of the various strains were examined on a variety of media. The appearances of well isolated colonies on agar and glucose agar plates, the characteristics of the growth on agar, glucose agar, broth, litmus milk and Dorset's egg medium, and the reactions produced with various sugars and alcohols were recorded.

THE BACILLUS OF MALIGNANT OEDEMA.

A pure culture of this bacillus was isolated in fifteen of the eighteen cases. All were cases in which considerable laceration and necrosis of tissue had occurred, and all were characterized by an offensive discharge. The degree and extent of the septic process varied considerably in the various cases of the series. Gas formation in the tissues was observed in four cases only, in three of which *B. aerogenes capsulatus* was also found. Of the fifteen cases three only were fatal—Cases 2, 16, and 17. In Case 2 there was a large gas-containing abscess of the arm, *B. aerogenes capsulatus* and *B. coli* were also present. Case 16 was one of tetanus. Case 17 was remarkable in that it was the only case of the series in which, although there was abundant gas formation in the tissues, *B. aerogenes capsulatus* was not found. Twelve cases of the fifteen in which *B. oedematis maligni* was found made good recoveries. There is apparently no reason to think that the presence of this micro-organism in a wound is necessarily of serious import. This conclusion is substantiated by the results obtained by the inoculation of animals. Its presence is an indication of the occurrence of gangrene, for it has probably little or no capacity for multiplication in living tissue. There appears to be no evidence that its presence is constantly associated with any particular group of signs or symptoms. Many of the films prepared from pus showed that this bacillus is readily taken up by the polymorphonuclear leucocytes.

Isolation of Pure Cultures.

The bacillus of malignant oedema grows extremely well on egg media. When but few are present in the discharge pure cultures are most readily obtained by freely inoculating the egg broth described in an earlier part of this paper and incubating in an anaërobic chamber for five to seven days. The *B. oedematis maligni* may then often be found in very large numbers and pure cultures may readily be obtained by plating on glucose agar.

Cultural Characteristics.

The appearances of a slope culture on Dorset's egg medium are very characteristic. A profuse creamy white growth appears within twenty-four hours. It increases rapidly and is at first raised above the surface of the medium. At the end of forty-eight hours, or even earlier, liquefaction is noticed. A clear fluid gradually accumulates in the lower part of the tube, in which float drops of oil. Liquefaction continues rapidly, and a bluish-black discoloration is a well-marked characteristic of *B. oedematis maligni* in this medium. The odour is extremely powerful and offensive; it is somewhat suggestive of gorgonzola cheese.

Films made from such a culture after incubation at 37° C. under anaërobic conditions for three days showed the following characteristics: The bacilli varied greatly in length; the larger forms, which were the more

numerous, were from 8 to 10 μ long and 0.6 μ wide. Of the shorter forms several were observed which measured about 2 μ in length and 0.6 μ in width. The majority of the bacilli occurred singly, but chains of two, four, and six members were met with. They were Gram-positive. Many of the bacilli contained spores which were of an oval shape and situated near to one end of the bacillus. Occasionally one bacillus appeared to contain two spores. A contained-spore of average size measured 1.5 μ by 1 μ . There were also a comparatively small number of free spores approximately 2 μ long by 1 μ wide. Films made from an egg culture after seven days' incubation showed numerous large, free oval spores, but very few bacillary forms. The bacilli present in these old egg cultures were Gram-negative.

Surface colonies on glucose agar plates showed a central opaque node from which radiated numerous long delicate hair-like processes. Streak cultures on glucose agar slopes showed a central opaque whitish line, with delicate hair-like lateral projections. Stab cultures in deep glucose agar tubes are not characteristic. Gas formation is well marked, but usually less than in cultures of *B. aerogenes capsulatus*. Profuse growth occurs along the deeper portions of the needle track. The lateral projections are seldom slender spikes, but more often in the form of disc or coin-shaped, circular, flattened projections. Very similar appearances are seen in stab cultures of *B. aerogenes capsulatus*.

Microscopical Appearances of Films made from a Glucose Agar Slope after Three Days' Incubation at 37° C., under Anaërobic Conditions.

The majority of the bacilli were Gram-negative. Variation in size was even more marked than in the case of the egg culture. The following dimensions, which are fairly representative, may be given:

Length	...	15 μ	Breadth	...	0.6 μ
"	...	8 μ	"	...	0.8 μ
"	...	3 μ	"	...	1.5 μ

Some of the shorter forms resembled oval cocci in outline. The majority were short single rods with rounded ends. There were also long chains of slender bacilli constituting delicate filaments, in which the subdivisions could be distinguished with difficulty or not at all. Spores were not found. After eight days' growth on glucose agar the appearances were not greatly different. All bacilli were Gram-negative and stained poorly. There were great variations in length. A few spores were seen.

The appearances of surface colonies on agar plates, slope cultures, and in deep stabs are similar to those observed with glucose agar. The growth is less profuse. The microscopical appearances of films from an agar slope after three days' incubation were similar to those seen in films made from the glucose agar culture of the same age. Spores, however, were fairly numerous. After eight days' growth on agar the films showed numerous short forms containing spores and a large number of free spores. The bacteria stained badly, and were Gram-negative.

On broth rapid growth occurred with the production of turbidity and an offensive odour. After about a week a deposit formed at the bottom of the tube. In broth cultures filamentous forms or chains were frequent, and attained a considerable length. Spore formations occurred readily, and the older cultures contained more spores than bacillary forms. In young broth cultures the majority of forms were Gram-positive, in older cultures Gram-negative.

After incubation in litmus milk for about seven days under anaërobic conditions very characteristic changes may be observed. When the anaërobic chamber is first opened the contents of the tube are seen in two layers. The upper layer is turbid but translucent, the lower layer consists of a bulky dirty white deposit. The odour is sour and offensive. If the culture is allowed to stand on the bench for twenty-four hours, colour returns to the upper layer. There are now three well defined layers. The uppermost layer has a reddish claret colour except at the actual air surface, where a poorly marked blue ring may often be seen. The middle layer is a colourless translucent, but slightly turbid, fluid. At the bottom of the tube is a bulky deposit. On shaking the tube the whole of the contents assume a claret colour. The fluid is amphoteric to litmus paper.

On inspissated serum a whitish growth appears, and the medium is slowly liquefied with the formation of a neutral or faintly alkaline fluid. There is no discoloration, but the colour of the culture is offensive.

B. oedematis maligni ferments glucose and maltose with the formation of acid and gas. Its action on these sugars is less rapid and less violent than that of *B. aerogenes capsulatus*. Some strains act very slowly, others show a well-marked acid reaction within forty-eight hours. Cane sugar, lactose, mannite, and dulcitol remain unaltered. After seven days' incubation under anaerobic conditions the appearance of a tube of serum water glucose or maltose is as follows: At the bottom of the tube is a considerable deposit consisting of flocculi and shreds of torn-up clot. The supernatant fluid is colourless and slightly turbid. At the surface a few shreds of clot of a bright red colour may be seen floating. On shaking, the entire contents of the tube assume a red colour. The culture has an odour suggestive of lactic acid. In the case of some of the strains of malignant oedema, the reaction of the glucose and maltose cultures after incubation was only slightly acid, and no clot was formed. In the majority of cases the sugar reactions were recorded shortly after the isolation of the pure culture, and the tests were repeated after the strains had been subcultured for several months. The strain isolated from Case 3 gave the same reactions in June as it had done shortly after isolation in the previous December.

B. AEROGENES CAPSULATUS.

This bacillus was isolated from 13 of the 18 cases. In all but one (No. 13) the bacillus of malignant oedema was also found. It was present in 3 of the 4 cases in which the presence of tetanus bacilli was demonstrated. It was present in 3 of the 4 cases in which signs of tetanus developed in the patient. It was present in 3 of the 4 cases in which gas was present in the tissues. Of the 13 cases only 2 were fatal, and in one of these death was due to tetanus.

Isolation.

This bacillus grows readily in broth and egg broth under anaerobic conditions. If the mixed culture which is prepared from the discharge be examined after forty-eight hours, large numbers of this bacillus are often found, and a pure culture may readily be obtained by plating on glucose agar.

Morphological and Cultural Characteristics.

In films made from the fluid present in the subcutaneous tissues of a guinea-pig which has succumbed to experimental inoculation, numerous short Gram-positive rods are seen. The majority occur singly and have square ends. The organism also occurs in pairs and chains of four. A capsule can be demonstrated. Many of the bacilli are seen within leucocytes. In some of the films made from the pus obtained from gangrenous wounds similar short definitely Gram-positive bacilli occurred. There were, however, other forms, somewhat larger, which stained unevenly by Gram's method. These bacilli exhibited a peculiar arrangement of Gram-positive stripes or granules on a Gram-negative ground. Both these and the Gram-positive forms were encapsulated.

B. aerogenes capsulatus grows excellently on Dorset's egg medium, although not so vigorously as does the bacillus of malignant oedema. The colonies are white, circular, and at first raised above the surface. After three or four days the medium is slowly eroded, and the colonies come to lie at the bottom of shallow saucer-like depressions. The digestion of the medium proceeds slowly, but nothing like the rapid liquefaction produced by *B. oedematis maligni* is seen. No discoloration of the medium occurs. Films prepared from a 48 hours' slope culture on Dorset's egg medium showed single short Gram-positive rods of fairly uniform length. There were a few long and comparatively slender forms and a few very short forms which resembled cocci. Spore formation occurs on this medium. The spores, which are oval in shape, distend the central portion of the bacillus, the remaining cytoplasm of the bacillus appearing as two caps, one at either end of the bacillus. Films prepared from old egg cultures showed great varieties in shape, size, and reaction to Gram's stain. Spores were numerous. On films made from egg medium long slender bacilli with a round

terminal spore are occasionally met with. These forms closely resemble the tetanus bacillus.

Isolated colonies on the surface of agar and glucose agar medium appear to the naked eye as circular colonies, 1 to 4 mm. in diameter, somewhat opaque, whitish in colour, and definitely thicker in the centre. Viewed under a $\times 10$ lens, the centre of the colony is seen to be opaque and raised up above a thin semitranslucent marginal zone, which often shows several rings. The margin is slightly wavy, and occasionally the irregularities of the outline are so well marked that an appearance of short offsets or processes is produced. Streak cultures on the surface of a glucose agar slope show a central opaque white line and a marginal somewhat translucent zone, which has a wavy outline. In glucose agar slabs the evolution of gas is commonly so violent that the wool plug and the greater part of the culture medium are expelled from the tube. Under less violent conditions the growth appears as an opaque white streak along the track of the needle, from which proceed flat coin-like outgrowths. The appearances of surface colonies on agar are similar to those on glucose agar, except that the growth is less vigorous. Films prepared from agar and glucose agar cultures of the same age show very marked differences. In films made from a 48 hours' agar culture short forms predominate. Many of the bacilli are very short, very broad, and present an almost square outline. Clostridial forms occur. The margins of many of the bacilli are indistinct and blurred. The bacilli occur singly. Both Gram-positive and Gram-negative forms may be found, but many show well-marked Gram-positive markings on a Gram-negative ground. Films prepared from a 48 hours' glucose agar culture showed considerable varieties in the length of individual bacilli, but long forms predominated. There were numerous pairs and short chains, and a few long chains of six, eight, or more bacilli. In older glucose agar cultures numerous club-shaped forms occurred. In neither agar nor glucose agar cultures were spores found.

Broth cultures are in no way characteristic. Uniform turbidity is seen in young cultures. Older cultures show a deposit and a comparatively translucent supernatant fluid. Films prepared from broth cultures showed numerous short broad rods. The majority occurred singly, but pairs and short chains were not uncommon.

On inspissated serum a greyish-white, not very profuse, growth makes its appearance. The surface of the medium is very slowly eroded. Films show forms similar to those seen in films prepared from egg medium. Spore formation occurs on this medium. Forms resembling the tetanus bacillus are occasionally met with.

The appearances of a litmus milk culture are well known. The production of acid and the formation of a clot are accompanied by a violent evolution of gas. After three or four days the tube is found to contain a clear acid translucent fluid and fragments of gas-contorted clot.

Sugar Reactions.—Violent fermentation occurs in media containing glucose and maltose with production of acid and much gas. Cane sugar and lactose are much less readily attacked, and the reaction in media containing these sugars is less acid. No reaction takes place with mannite, dulcitol, and inulin. The reactions can be demonstrated by the use of the ordinary peptone-sugar media or by Hiss's serum water sugar media. In the latter media the production of acid is accompanied by the formation of a clot, the disruption of which affords satisfactory evidence of the evolution of gas. The torn and lacerated clot, and the clear, strongly acid fluid which are found in the serum maltose tubes and serum glucose tubes present very characteristic appearances. The sugar reactions of *B. aerogenes capsulatus* were found to be quite constant, and were the same in all the strains examined.

INOCULATION EXPERIMENTS.

The results obtained by the inoculation of the mixed growth contained in the original broth cultures yielded very variable results.

Inoculation experiments were undertaken with eight strains of *B. oedematis maligni* and with five strains of *B. aerogenes capsulatus* in pure culture. In the first series of experiments the inoculated material consisted of either a broth culture, or an emulsion in broth of several loopfuls of the surface growth from a Dorset's egg or agar slope. The injections were subcutaneous. Thirteen

guinea-pigs and two rabbits were inoculated with cultures of *B. oedematis maligni*. One of these animals died twenty-four hours after inoculation. The cause of death, which could not be ascertained, was probably not the inoculation, for the site of the injection showed only a slight inflammatory reaction. The remainder of the animals survived and remained in good health for one month after the inoculation. In a few cases there were signs of illness during the first forty-eight hours, and examination revealed a slight swelling at the site of inoculation. Recovery was in these cases rapid and apparently complete. In the first series of experiments with *B. aerogenes capsulatus* twelve guinea-pigs and two rabbits were used.

Two guinea-pigs which had been inoculated subcutaneously at 5 p.m. on November 25th with 2 c.cm. of a broth culture of *B. aerogenes capsulatus* from Case 2, were found dead at 9 a.m. on November 26th. Emphysema was found at site of inoculation and the skin had been separated from the deep tissues over the greater part of the trunk. The subcutaneous tissues were infiltrated by a blood-stained fluid, in which floated globules of fat. Gas was present in the subcutaneous tissues, but not in the organs of the thorax and abdomen. Films made from the site of inoculation contained large numbers of *B. aerogenes capsulatus*, and a pure culture of this bacillus was obtained from the exudate. The heart blood was sterile.

On December 11th a guinea-pig received a subcutaneous injection of 1 c.cm. of a broth culture of the same strain (Case 2). The animal was found dead on December 15th. Necrosis of tissue was found at the site of the injection, and films showed *B. aerogenes capsulatus* and numerous streptococci. All the organs of the body and the serous membranes were engorged with blood. The lungs showed patchy consolidation. Films made from the lung showed streptococci only. The heart blood was sterile.

On December 11th a guinea-pig received a subcutaneous injection of 1 c.cm. of a broth culture of *B. aerogenes capsulatus* (Case 3). Slight local swelling and gas formation followed. The animal had apparently completely recovered after forty-eight hours. It died on December 18th. At the post-mortem examination an acute volvulus of the small intestine was found.

In two additional experiments with pure cultures from strain No. 2 the animals remained in perfect health for two months. Similar experiments were performed with pure cultures from Cases 10, 12, 13, and 14. In each case two guinea-pigs were inoculated. One of the animals inoculated with Strain 13 died after twenty-four hours from typical gas gangrene. The remaining seven animals were kept under observation for more than a month and showed no evidence of infection.

Two rabbits received a subcutaneous injection of 2 c.cm. of a pure culture of Strain 2. After twenty-four hours there was a slight local swelling and the animals appeared to be slightly unwell. Recovery had taken place at the end of forty-eight hours and no further symptoms were observed.

The conclusion arrived at from these experiments was that the subcutaneous injection of pure cultures of *B. aerogenes capsulatus* may occasionally give rise to fatal gas gangrene. With possibly one exception, all the experiments with pure cultures of *B. oedematis maligni* yielded negative results.

The results obtained by animal inoculation with pure cultures accord well with the evidence afforded by the clinical findings in the various cases above recorded. Both *B. oedematis maligni* and *B. aerogenes capsulatus* were present in the majority of the cases; but three only of the eighteen patients died, and in four cases only was gas formation observed in the tissues. We must assume that neither of these micro-organisms is truly parasitic—that is to say, neither bacillus is capable of multiplication in living tissue. In blood clot, in dead tissue, and fragments of foreign bodies which are so often present in wounds, they are capable of active growth and multiplication. Here they produce, by ferment action, poisonous substances, possibly organic acids,* which, in circumstances favourable to them, diffuse out into the surrounding living tissue. The result is that the adjacent tissues die and are then invaded by the micro-organisms. In this fashion the gangrenous area increases in size, successive zones of tissue being first poisoned and then invaded by the saprophytes.

Acting on the assumption that the pathogenic action of these micro-organisms might be dependent on the action

of the organic acids which they produce, the following experiments were undertaken:

B. oedematis maligni (Strain 3) and *B. aerogenes capsulatus* (Strain 2) were subcultured on agar, broth, Dorset's egg medium, and on serum water, cane sugar, glucose, lactose, maltose, mannite, and dextrose. With *B. aerogenes capsulatus* marked acid reactions were obtained with glucose and maltose, and less marked reactions with cane sugar and lactose. With *B. oedematis maligni* the media containing glucose and maltose became slightly acid. A portion of each medium was injected into a guinea-pig. In the case of the fluid media the quantity inoculated was 2 c.cm.; in the case of the solid media one loopful of the growth was emulsified in 2 c.cm. of broth. It was thought that the cultures which contained acid might prove the more pathogenic. The result of the experiment was negative. In no case was gangrene produced, and, with the exception of slight and transient symptoms, all the animals remained in perfect health. In a third series of experiments guinea-pigs were inoculated with various strains of *B. oedematis maligni* and *B. aerogenes capsulatus* mixed with a recently isolated strain of *Staphylococcus aureus*. It was thought that the anaerobic bacteria might gain a hold in the tissue damaged by the action of *Staphylococcus aureus*.

INOCULATION EXPERIMENTS.

Series 3.

Experiment 1.—*B. oedematis maligni*, Strain 8, alone. Inoculated on May 27th. No symptoms. Animal alive and well on July 1st.

Experiment 2.—*B. oedematis maligni*, Strain 8, + *Staphylococcus aureus*. Inoculated on May 27th. No symptoms. Animal alive and well on July 1st.

Experiment 3.—Same as Experiment 1.

Experiment 4.—*B. oedematis maligni*, Strain 8, + *Staphylococcus aureus*. Inoculated on May 27th. Animal ill, with local swelling, on May 29th. Died on June 2nd; gangrene of subcutaneous tissues, blood-stained rancid effusion. Films showed pus cells, cocci, and *B. oedematis maligni*.

Experiment 5.—Same as Experiment 1.

Experiment 6.—*B. oedematis maligni*, Strain 8, + *Staphylococcus aureus*. Inoculated on May 27th. No local symptoms. Died on June 14th. No lesion at site of inoculation. No obvious cause of death.

Experiment 7.—*B. oedematis maligni*, Strain 2, alone. Inoculated on May 27th. Died on June 12th. No local lesion; no obvious cause of death.

Experiment 8.—*B. oedematis maligni*, Strain 2, + *Staphylococcus aureus*. Inoculated on May 27th. Died the following day. Swelling and oedema at site of inoculation. Film showed numerous cocci and *B. oedematis maligni*.

Experiment 9.—*B. oedematis maligni*, Strain 15, alone. Inoculated on May 27th. No symptoms. Alive and well on July 1st.

Experiment 10.—*B. oedematis maligni*, Strain 15, + *Staphylococcus aureus*. Inoculated on May 27th. Died on June 21st. No local lesion; no obvious cause of death.

Experiment 11.—Same as Experiment 9.

Experiment 12.—*B. oedematis maligni*, Strain 15, + *Staphylococcus aureus*. Inoculated on May 27th. On May 29th there was local swelling, which was marked on the following day; animal very ill. On the 31st it was much better. It eventually recovered, and was quite well on July 1st.

Experiment 13.—*B. aerogenes capsulatus*, Strain 2, alone. Inoculated on May 27th. On the 28th there was local swelling, and the animal was ill. On the 29th the swelling had disappeared, and the animal was well. It recovered completely, and was alive and well on July 1st.

Experiment 14.—*B. aerogenes capsulatus*, Strain 2, + *Staphylococcus aureus*. Inoculated on May 27th. On May 29th there was local swelling; animal slightly unwell. It recovered completely, and was alive and well on July 1st.

Experiment 15.—*B. aerogenes capsulatus*, Strain 3, alone. Inoculated on May 27th. Died on June 18th. No local lesion; no obvious cause of death.

Experiment 16.—*B. aerogenes capsulatus*, Strain 3, + *Staphylococcus aureus*. Inoculated on May 27th. No symptoms. Animal alive and well on July 1st.

Experiments 17 and 18.—Controls: *Staphylococcus aureus*, alone. Inoculated on May 27th. No symptoms. Animals alive and well on July 1st.

The results obtained in the above series of inoculations were not very conclusive. Possibly a more interesting result might have been obtained by the use of a more virulent culture of *Staphylococcus aureus*. Of the eighteen animals, two died with typical gangrene around the site of inoculation. Both of these animals (No. 4 and No. 8) had been inoculated with a mixture of *B. oedematis maligni* and *Staphylococcus aureus*. The control animals (No. 3 and No. 7), which were inoculated with the same strains of *B. oedematis maligni* and *Staphylococcus aureus*,

* After our paper had been sent to press there appeared in the BRITISH MEDICAL JOURNAL of December 4th a Note on a Supposed Soluble Toxin, Produced in Artificial Culture by the Bacillus of Malignant Oedema, by G. Barger and H. H. Dale. These workers have been able to prove that the "poisonous substances," the presence of which we suspected, are ammonium salts. The well-marked proteolytic activity of *B. oedematis maligni* is referred to in our paper.

did not develop gangrene. On the other hand, two other guinea-pigs (No. 2 and No. 6), which were inoculated with a mixture of *Staphylococcus aureus* with the same strain of *B. oedematis maligni* as was used in Experiment No. 4, did not develop gangrene. In the case of several of the patients recorded in this series pieces of clothing were removed from the depth of the wound. It is presumed that such fragments, which must be often covered with mud and impregnated with anaërobic bacteria or their spores, may often form the starting-point of gangrene.

In the following four experiments an attempt was made to reproduce these conditions by the introduction of a foreign body heavily charged with anaërobic bacteria. Splinters about $\frac{1}{4}$ in. long were prepared from wooden matches and impregnated with cultures of *B. aerogenes capsulatus* and *B. oedematis maligni*. They were then introduced into the subcutaneous tissues of guinea-pigs.

INOCULATION EXPERIMENTS.

Series 4.

Experiment 1.—*B. oedematis maligni*, Strain 8. Splinter inserted on June 26th. The animal was well on June 28th. Marked swelling developed around the splinter on the 29th, and on the 30th the animal was very ill. It subsequently recovered completely.

Experiment 2.—*B. oedematis maligni*, Strain 8. Splinter inserted on June 26th. On June 28th there was marked swelling around the splinter; animal ill. It died the following day. Inflammation and oedematous infiltration around splinter.

Experiment 3.—*B. aerogenes capsulatus*, Strain 2. Splinter inserted on June 26th. The animal was well until June 30th, when marked swelling developed around the splinter. Animal very ill, but subsequently recovered completely.

Experiment 4.—*B. aerogenes capsulatus*, Strain 2. Splinter inserted on June 26th. No symptoms developed.

The series of inoculation experiments with these strains of *B. oedematis maligni* and *B. aerogenes capsulatus* led to the following conclusions:

1. The subcutaneous injection of pure strains does not, as a rule, produce any sign or symptom of disease.
2. If the tissues are damaged by a parasitic micro-organism, foreign body, or other irritant, the introduction of *B. aerogenes capsulatus* or *B. oedematis maligni* may, in a comparatively small number of cases, lead to local gangrene and the death of the animal.

TETANUS.

No. of Case.	Symptoms of Tetanus in Patient.	Prophylactic Injection of Antiserum.	Presence of <i>B. tetani</i> Proved by Inoculation.	No. of Days required for Bacteriological Diagnosis.	Other Anaërobic Bacteria Present.
No. 1	+	0	0	—	<i>B. oedematis maligni</i> , <i>B. aerogenes capsulatus</i> .
No. 6	+	0	+	19	<i>B. oedematis maligni</i> .
No. 10	0	+	+	6	<i>B. oedematis maligni</i> .
No. 14	0	+	+	15	<i>B. oedematis maligni</i> , <i>B. aerogenes capsulatus</i> .
No. 15	+	0	0	—	<i>B. oedematis maligni</i> , <i>B. aerogenes capsulatus</i> .
No. 16	+	0	+	13	<i>B. aerogenes capsulatus</i> .

The methods which were employed with the object of establishing a bacteriological diagnosis of tetanus fall under three headings:

1. The microscopical examination of (a) films from the discharge from the wound, (b) films from the original mixed culture prepared by the inoculation of broth with the discharge.
2. The inoculation of guinea-pigs with the original mixed culture.
3. The isolation of *B. tetani* in pure culture.

1. Examination of Films.

In none of these cases were bacteriological forms resembling *B. tetani* found in films made from the discharge. In the films made from mixed broth cultures tetanus forms were observed in Cases 6, 10, 14, and 16, and also in Case 1. By the expression "tetanus form" is implied a slender

Gram-positive rod bearing a large absolutely terminal round spore. Microscopical examinations of films from the original broth culture of Case 15 were made on numerous occasions, but no forms resembling tetanus were observed.

In Cases 6 and 16 tetanus forms could not be demonstrated until the mixed culture had been incubated under anaërobic conditions for a relatively long period. Unfortunately no systematic attempt was made to record the number of days which elapsed in each case before bacteria resembling tetanus appeared in the broth cultures.

The following observations were, however, recorded:

	Tetanus Forms not present after	Tetanus Forms present after
Case 1	...	2 days
Case 6	6 days	17 days
Case 10	...	4 days
Case 14	3 days	8 days
Case 16	5 days	10 days

It appears that:

1. Tetanus bacilli are often not found in films made from the discharge from the wound.
2. A long time may elapse before they appear in cultures in sufficient number for microscopical recognition.

A more serious objection to the microscopical method of diagnosis is the fact that closely similar, if not indistinguishable forms, occur in films made from pure cultures of *B. oedematis maligni* and *B. aerogenes capsulatus*. On the other hand, a pure culture of *B. tetani* may often contain many bacilli which are in no way characteristic in appearance. In the absence of other evidence, the discovery of tetanus-like bacilli in films is not satisfactory evidence of the presence of *B. tetani*. The presence in a film of numerous slender Gram-positive bacilli, bearing a large round terminal spore, is, of course, a suspicious circumstance, and may justify a preliminary opinion. The microscopical evidence is, however, not sufficient, unless supported by the result of an inoculation experiment.

2. Inoculation of Mixed Broth Culture.

The presence of *B. tetani* was demonstrated in Cases 6, 10, 14, and 16 by injecting a guinea-pig with the original mixed broth culture.

One or two cubic centimetres of the culture were injected beneath the skin on the left side of the chest. After a variable interval—usually two or three days—slight stiffness of the forelimb nearest to the site of inoculation was observed. Within a few hours the limb became rigid in a position of extreme extension. Subsequently a condition of extensor spasm spread to the other limbs and the muscles of the neck and back. Death usually occurred within twenty-four to forty-eight hours after the first onset of symptoms. In the majority of cases there was little or no evidence of inflammation at the site of inoculation. The method is a satisfactory one, and yields results which are not open to doubt. The disadvantage is the considerable interval that may elapse before a demonstrable amount of toxin is produced.

3. Isolation of Pure Cultures.

In Cases 6, 10, 14, and 16 tetanus bacilli were found in large numbers in the original mixed broth cultures, and the inoculation of these broth cultures produced characteristic symptoms in animals. In only one of these cases was the tetanus bacillus isolated in pure culture, in the other three cases repeated attempts ended in failure. Agar, glucose agar, and blood agar plates were employed; colonies of *B. oedematis maligni* were readily obtained, but not of tetanus. Fractional heating was employed in the hope that the spore of tetanus might prove more resistant than that of malignant oedema. No success was obtained in this way. A pure culture was eventually obtained by the inoculation of glucose agar plates with the mixed broth culture from Case 16.

Cultural Characteristics.

The surface colonies on glucose agar plates varied greatly in size. They were round, opaque, and rather white. Under a hand lens they showed a central portion which was thicker and made up the greater part of the colony, surrounded by a thin, translucent margin, which had a wavy outline.

With higher magnification the centre of the colony was seen to be granular, while the margin showed delicate rounded projections. In older cultures numerous small circular opaque nodes or thickenings appeared on the colony. These may perhaps be described as secondary colonies. Other colonies showed large branching outgrowths, which were beautifully rounded, and somewhat suggested the contour of an oak-leaf. Streak cultures on glucose agar slopes showed a central thick opaque line and a broad, translucent margin, showing branching and rounded processes. The appearance of agar cultures was similar, but the growth was not so vigorous. Broth cultures became turbid with the formation of a deposit. The growth on Dorset's egg medium was not very vigorous, but resembled that on glucose agar. Flat white colonies appeared, which after a time came to lie in shallow depressions in the medium. No change was produced in litmus milk. The sugar reactions were examined on several occasions with Iliss's serum water, and with peptone water media. This strain produced no change on cane sugar, glucose, maltose, lactose, mannite, dulcitol, and inulin.

The microscopical appearances of films from various culture media were recorded. As only one strain was examined, the results have little value.

The appearance of bacilli grown on agar, glucose agar, and broth accorded with the description usually given of this micro-organism. The bacilli were slender, showed considerable variations in length and size, and had rounded ends. The majority occurred singly. Many bacilli contained spores, the majority of which were terminal, round, and very large. In a very few bacilli the spores were oval in shape and were either in the centre of the bacillus or near but not actually at one end. These forms showed a close resemblance to the appearances commonly met with in cultures of *B. oedematis maligni*. In a few of the bacilli there was an appearance of granules similar to that seen in films of *B. aerogenes capsulatus*. Bacilli which appeared to have two terminal spores occurred occasionally. Films made from a three days old culture on Dorset's egg medium were distinctly different in appearance. The majority of the bacilli appeared to be broader than those grown on agar. Although in many bacilli the spore was terminal, there were many others in which it was oval and occupied a central position. Apart from the spore-bearing forms, bacilli occurred in which the poles stained faintly, while the centre appeared as a solid deeply stained band. In other bacilli the arrangement was reversed, and a bipolar effect was produced. Possibly these appearances represent early stages of spore formation.

Inoculation Experiments.

Eleven guinea-pigs and 2 rabbits were inoculated from a ten-day-old pure broth culture of this strain. Tetanus developed in 4 of the guinea-pigs. The other 7 guinea-pigs and the 2 rabbits showed no evidence of the disease.

Remarks on above Cases of Tetanus.

It is perhaps worthy of note that the two patients who did not suffer from tetanus although the presence of tetanus bacilli was demonstrated in the wounds had received a prophylactic injection of antitoxin. The four patients who developed signs of tetanus did not have a prophylactic injection. In Cases 10, 14, 15, and 16, fragments of clothing were removed from the wound. It may be assumed that the presence of fragments of cloth, saturated with the discharges of a gangrenous wound, must form a peculiarly suitable nucleus for the growth of tetanus bacilli.

The results above recorded lead to the conclusion that the microscopical examination of films made from the discharge from a wound may be of little value in the diagnosis of tetanus. Satisfactory results may be expected from the injection of guinea-pigs with broth which has been inoculated from the discharge. Prolonged incubation may be necessary before a positive result is obtained. Under these circumstances the patient may develop tetanus before a diagnosis is arrived at in the laboratory. Now the tetanus bacillus belongs to the same group of bacteria as do *B. aerogenes capsulatus* and *B. oedematis maligni*, and the demonstration of the presence of these two micro-organisms is a relatively simple and rapid matter. All three micro-organisms are of intestinal origin and are apt to be found in polluted soil. All three are no doubt frequently simultaneously introduced into a wound, and all three require the same conditions for growth and multiplication. The presence of either *B. aerogenes capsulatus* or *B. oedematis maligni* might with great advantage be taken as an indication for one or more

prophylactic injections of antitetanus serum. In two at least of the above cases the first symptoms of tetanus followed immediately on an operation, and it appears to us that antitetanic serum should be injected as a prophylactic measure before any considerable operative procedure is attempted on a patient with a gangrenous wound.

Since the conclusion of this investigation two other cases have been observed in which the onset of tetanic symptoms was apparently determined by operative procedure.

TREATMENT.

In the summary of our cases no reference has been made to treatment, and we did not in the first instance contemplate any reference to the matter. It may, however, be remarked that the results obtained were, considering the gravity of the majority of the cases, satisfactory; and as the bacteriological findings exhibited a marked uniformity, a brief note on the general lines of treatment may be of some interest. Cases 17 and 18 received continuous irrigation with hypertonic saline. In the other sixteen cases treatment consisted of irrigation with hydrogen peroxide (5 to 10 vols.) and fomentations of hot sterile isotonic saline solution (0.9 per cent.) or boracic lotion. In the later stages, when the wounds had cleaned up, lotio rubra and gauze dressings were employed.

After the conclusion of this investigation, irrigation with hypertonic saline solution was employed in more than 100 cases which presented similar features to those above recorded. The method caused considerable discomfort to the patients, and did not yield results in any way superior to those obtained by the use of older methods of treatment. In this connexion it may be noted that active phagocytosis was often observed in films prepared from the discharge of wounds which had been treated by the usual methods.

B. aerogenes capsulatus and *B. oedematis maligni* were frequently isolated from wounds in which no gas was produced and the gangrenous area showed no tendency to spread. It may be inferred that the conditions which enable these micro-organisms to exercise any serious influence in the course of a case are only exceptionally present. These micro-organisms are essentially saprophytes, and are relatively, if not entirely, innocuous to undamaged living tissues. The presence of dead tissue or fragments of clothing and the retention of discharge or blood clot within a wound provide these micro-organisms with a favourable culture medium. It is of the utmost importance that wounds containing anaerobic bacteria should be freely opened up and thoroughly drained.

SUMMARY.

1. The series comprises 18 cases of gangrenous wounds, of which 3 only were fatal. Included in this total are 4 cases of tetanus (1 of which was fatal), and 4 cases of gas gangrene (2 of which were fatal). Of the 18 cases *B. oedematis maligni* was found in 15 and *B. aerogenes capsulatus* in 13.

2. *B. aerogenes capsulatus* and *B. oedematis maligni* are apparently possessed of powerful enzymes. The former is peculiarly able to attack carbohydrates, the latter proteins. Dorset's egg medium is an admirable medium for both micro-organisms.

3. The shape, size, staining reactions, and capacity for spore formation of these bacilli are profoundly influenced by the nature of the culture medium.

4. On Dorset's egg medium the majority of the bacilli are typical in shape, uniform in size, and Gram-positive. On media which contain a carbohydrate, from which the bacilli can form acid, growth is at first rapid and vigorous, but after a few days the bacilli become atypical in appearance, vary greatly in size, and the majority are Gram-negative.

5. *B. aerogenes capsulatus* forms spores on Dorset's egg medium and inspissated serum, but not on media in which an acid reaction is produced. *B. oedematis maligni* forms spores less readily in acid media.

6. The presence of *B. aerogenes capsulatus* and *B. oedematis maligni* is not necessarily associated with the development of gas in the tissues.

7. *B. oedematis maligni* and *B. aerogenes capsulatus* are essentially saprophytes. They have little or no power to multiply in living tissue. In dead tissue they grow

rapidly and produce poisonous substances, by which the adjacent living tissue is destroyed and rendered a suitable medium for the further multiplication of these bacilli.

8. *B. tetani* was not found in films made from the discharge in any one of the six cases of this series in which it was present.

9. The recognition of *B. tetani* by purely microscopical methods is complicated by the fact that slender Gram-positive rods bearing an absolutely terminal spore may be occasionally found in pure cultures of *B. oedematis maligni* and *B. aerogenes capsulatus*. Moreover, pure cultures of tetanus bacilli, especially cultures on egg medium, contain many atypical forms.

10. If broth is inoculated with material from the wound in a case of tetanus, and incubated under anaërobic conditions, the presence of *B. tetani* can often be satisfactorily demonstrated by animal inoculation. Such a broth culture should be examined at intervals, and two or three weeks may elapse before *B. tetani* can be demonstrated.

11. The presence of *B. tetani* was demonstrated in the discharge from the wounds of two patients who did not develop signs of tetanus. Both had received prophylactic injections of antitetanus serum.

12. The discovery of *B. tetani* in the wounds of a patient who had not developed tetanus would obviously be an indication for one or more prophylactic injections of antitetanic serum. But the practical utility of such a procedure is limited by the difficulty and delay which attend the bacteriological recognition of this bacillus. Now, *B. tetani* belongs to the same group of anaërobic bacteria as *B. aerogenes capsulatus* and *B. oedematis maligni*. All three have probably a common source, and the conditions favourable to their growth within a wound are probably identical. The demonstration of either *B. oedematis maligni* or *B. aerogenes capsulatus* is a relatively simple matter and does not involve much delay. The discovery of either of these bacilli might with advantage be followed by a prophylactic injection of antitetanic serum.

13. A prophylactic injection of antitetanic serum should be given before any considerable operation is performed on a patient with a gangrenous wound.

THE

TREATMENT OF ACUTE TOXAEMIA SECONDARY TO GAS GANGRENE BY THE INTRAVENOUS INJECTION OF A SOLUTION OF HYPOCHLOROUS ACID.

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(Report to the Medical Research Committee.)

It has been our experience that soldiers whose wounds are infected by a gas-producing organism sometimes suffer from a condition which can only be described as an intense toxæmia and which frequently proves fatal.

Such cases present features as follows: The patient is admitted to hospital suffering from a wound of one of the extremities; there is extensive infection by a gas-producing organism, and it is obvious that the infected part must be amputated as soon as possible.

In some of these cases, although the operation is carried out, it is found that the general symptoms and signs of toxæmia do not abate but develop and deepen till death ensues; the following two cases are illustrative of these facts:

CASE A.

A sergeant-major, admitted on October 15th, 1915, suffering from a gunshot wound of the left upper arm a short distance below the shoulder-joint. There was a complicated compound fracture of the humerus, and the

brachial artery was divided. The wound was infected by a gas-producing organism, and the infection was spreading in the surrounding tissues. His general appearance was that of a man who was suffering from severe hæmorrhage; he had an intense grey pallor, and this was the more extraordinary because inquiry showed that the loss of blood had not been excessive; his features were distressed. The pulse was 130, the temperature (see Chart) 97° F., and the respirations 24. He was suffering from the condition of general toxæmia which so closely resembles shock. It was decided that amputation was necessary, but the operation was delayed for some time in the hope that matters might improve. After some hours there was no improvement and disarticulation through the shoulder-joint was carried out.

At the operation the fact was confirmed that the loss of blood was not severe, for the vessel at its site of division was twisted into a fibrous cord.

The post-operation history was disappointing, not as regards the local condition—for no trace of infection developed in the stump—but in regard to the general condition. The pallor remained, and, if anything, deepened; there was a faint underlying tinge of jaundice, there were profuse perspirations and occasional rigors, the pulse-rate increased to 150, the respirations quickened from 24 to 60, unconsciousness developed, and he died on the fourth day following the operation. After the amputation of the diseased limb there was no local site which could be regarded as the source and origin of the poisoning from which the man was suffering. A process of reasoning led to the conclusion that there was proceeding either a septicæmia or a toxæmia which had originally been instituted from the infected wound. A blood examination was carried out by Captain Ellis, but no organism could be isolated. The process was apparently, therefore, a toxæmia.

CASE B.

A private, admitted on October 30th, 1915, suffering from a gas-infected wound of the right leg. Generally he presented an appearance very similar to that of Case A—an intense pallor as though from severe loss of blood, restlessness and sweating; the temperature was 97° F., and the pulse 140. The right limb below the knee was extensively infected with a gas-producing organism; the bones were not injured, but the main blood vessels were involved. It was decided that amputation was necessary, and this was carried out through the knee-joint under spinal anaesthesia. After the operation the stump did well, but the general condition was far from satisfactory; the temperature rose and remained high; the pulse increased in rapidity and became weaker. On the morning of the second day after the operation the patient seemed better, but immediately after being dressed he collapsed and died.

We have chosen these cases at random from amongst others. To our minds they are illustrations of cases suffering from intense toxæmia. The removal of the original and local source of the toxæmia was not sufficient to counteract the malady, for the poison had acquired so firm a hold that its continued activity led to the death of the patients. The counteraction of the toxæmia formed a very distinct problem, and while it was under consideration Professor Lorrain Smith of Edinburgh suggested to us that eusol, which contains 0.5 per cent. hypochlorous

CASE A.—Gas gangrene: disarticulation through the shoulder-joint on October 15th. D, Death.

CASE B.—Gas gangrene: amputation through the knee-joint on October 30th. A, Admission; O, operation; D, death.