NOTES UPON CERTAIN ANAEROBES ISOLATED FROM WOUNDS.\textsuperscript{1}

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The following is a brief account of certain anaerobes isolated from gangrenous wounds. The cultures were derived from cases which occurred in Flanders, from November 1914 to February 1915. I am indebted to Major Rowland, R.A.M.C., and to Lieutenants Petrie and Henry, R.A.M.C., for their kindness in sending me this material. Three cultures were also generously placed at my disposal by Mr. Goadby; these were from cases under his care at the Herbert Hospital, Woolwich.

The investigation recorded here was begun by Dr. Arkwright and Dr. C. J. Martin, and was left in my hands when they were called away to more important work. The general lines of the inquiry were laid down by them, and the work owes its inception and a good deal of its subsequent carrying out either directly or indirectly to their interest and experience.

The material investigated was derived from forty-two cases of wound gangrene. In addition, the following strains were studied so as to afford a basis of comparison:—

Two malignant edema laboratory cultures.
One malignant edema strain obtained from a sheep, and kindly passed on to me by Mr. Buxton.
A tetanus culture from Dr. R. O'Brien.
A culture derived from Messrs. Parke Davis' "Blacklegoid" vaccine, which proved to be \textit{B. oedematis maligni} (Koch).
A culture of the \textit{Vibrio septique} kindly sent me by Dr. Roux from the Pasteur Institute, Paris.
Two \textit{B. perfringens} (\textit{B. aerogenes capsulatus}) strains isolated from milk. \textit{B. perfringens} and malignant edema (Koch) were both cultivated by animal passage from a small sample of trench earth obtained from Flanders in November 1914.

During the progress of the investigation it became clear that a uniform set of growth tests was of great advantage in distinguishing

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and classifying the various forms. The following media were found useful for this purpose:—

Litmus milk.
Litmus milk, with the addition of precipitated chalk.
Inspissated horse or ox serum.
Nutrient gelatin.
Cooked meat medium.
Alkaline egg fluid.
Alkaline egg fluid added to broth in the proportion of 1 to 5.
Glucose broth.
Glycerin broth.
Inulin broth.
Starch broth.

Glucose agar is useful for stabs, shakes, and plates, and sterile blood broth is a valuable medium for growing the anaerobes direct from the animal body. It may be noted that if inspissated horse or ox serum is difficult to obtain, Dorset's egg medium can be substituted. Those organisms which liquefy inspissated serum also liquefy Dorset's egg medium. The recipe for such of the above media as are not in general use are given in an Appendix (p. 348).

These or somewhat similar media have long been in use for the anaerobes, but they have not been very systematically applied as definite diagnostic tests until v. Hibler's work (1908). The series of media proposed follow v. Hibler's in their main lines, and one would like to suggest that the general application of some such series of diagnostic tests in describing the spore-bearing anaerobes would clear up many of the present confusions and obscurities and would form the basis of a sound classification. The most important members of the series are milk, inspissated serum, nutrient gelatin, and the cooked meat medium.

Three different anaerobic species were isolated from the wound material, namely:—

(1) Bacillus perfringens (B. aerogenes capsulatus, Welch and Nuttall; B. Welchii, auctt.; B. phlegmone emphysematose, Fraenkel).
(2) Bacillus of malignant edema (Koch).
(3) An endsporing bacillus which does not liquefy gelatin, agreeing very closely with v. Hibler's bacillus No. ix, described in his book “Die pathogenen Anaeroben,” 1908, and also allied to Rodella's bacillus iii. Rodella's description (1902) is, however, not sufficiently full to allow of a certain diagnosis. I have adopted the designation Hibler ix (H ix) for this organism.

The justification for the application of these names will be considered in a later part of the paper.

I propose to give the characters and reactions of these three anaerobic bacilli before going into the question of their incidence and the great difficulties experienced in obtaining pure cultures in the very frequent cases in which more than one anaerobe was present. It suffices for the moment to say that the most meticulous care was exercised in finally getting a certain number of strains in pure culture.
(1) **Bacillus perfringens.**—This is a non-motile Gram-positive organism, nevertheless in cultures which have been growing for some time (especially in fluid media) many Gram-negative individuals may be seen. The colonies on surface plates of glucose agar are smooth and round and raised above the surface, they do not grow down into the medium and do not develop filaments. In deep plates or in shakes they are lenticular or shaped like a whetstone. Glucose agar is a good medium for plates, as growth is rapid; it must, however, be noted that after a relatively short period (three to seven days) the organisms may die owing to the excessive amount of acid produced by the fermentation of the glucose. When *B. perfringens* is obtained direct from the animal body, it is found to have a well-defined capsule; this is not to be observed when the organism is growing upon culture media. It can, however, be demonstrated at any time if the bacilli are placed in serum (human and ox were those actually used), or in a weak solution of sodium hydrate. The capsule is an envelope in which the bacillary body lies in much the same way as a finger within a glove; in preparations made with the weak solution of sodium hydrate individuals can be seen with the bacillary body partially or wholly extruded from the capsule. Cilia or flagella could not be demonstrated at any time.

When grown on the cooked meat medium a bright pink colour is produced, and there is a very considerable development of gas; the medium is turned acid, and there is a sourish smell, but no odour of putrefaction. Gelatin is liquefied after a few days' growth. Inspissated serum is not liquefied even after months of incubation; there is a tendency to grow in long filaments upon this medium. In milk there is formed a tough clot which is torn by the active production of gas; there is a strong smell of butyric acid, the reaction is acid, and growth stops after a few days. A milk culture of *B. perfringens*, kept under anaerobic conditions, dies as a rule in ten to fourteen days. There is no obvious digestion of the clot, nor have I ever seen digestion to occur in a pure culture of *B. perfringens* grown in chalk milk, though Tissier and Martelly (1902) describe the complete peptonisation of the clot in this medium. Glucose broth shows active fermentation, both acid and gas being formed. There is a tendency to ferment both inulin and glycerin, but this character is somewhat variable. Starch broth is not fermented, although very good growth is obtained in this medium. When grown on alkaline egg fluid the medium is rendered more opaque, but a definite flocculent precipitate is not formed. *B. perfringens* does not form spores under cultivation, except in media rich in protein and poor in fermentable sugar. My own experience agrees with that of von Hibler and other workers on this point. I have found that spores are formed in the course of three or four days' growth upon inspissated serum; they are also formed regularly in the alkaline egg medium, and exceptionally upon cooked meat medium. I have never found that
spores were formed in glucose broth or upon glucose agar. The spores are central or subterminal and relatively large; they are oval in shape; the bacillary rod loses its power to retain the Gram-stain, and the spore is set free apparently as soon as its development is complete.

Of the seven strains of *B. perfringens* derived from wounds, which were finally isolated in pure culture, six were quite definitely pathogenic for guinea-pigs, though they were not all quite equally virulent. For instance, K 13b, which was derived from a single bacillus, had a virulence a little below the average of the other pathogenic strains. The seventh strain (K 711) did not appear to be seriously pathogenic; this was derived from a case of generalised gas gangrene, the original material also containing an organism of the proteolytic group which belonged to the malignant cedema type. An eighth strain derived from wounds was also pathogenic, but it was found later to contain H IX as well, so these results were discarded. Two *perfringens* strains isolated from milk were tested in regard to their pathogenicity; of these one strain (milk, July 1) produced a quite typical fluid cedema culminating in death in fifteen hours, whereas the other (milk, July 3) when injected in the same dose occasioned only a considerable swelling of the inoculated limb, from which the pig recovered after two days' illness.

The questions as to what constitutes a lethal dose, the course of the illness, and the number of spontaneous recoveries were gone into in some detail. The virulence of a given strain was found to vary a little according to cultural conditions, but all the pathogenic strains retained their pathogenicity over months of cultivation substantially unchanged. A period of quiescence in the sporing state in the alkaline egg medium was found, when the strain was subcultured into broth, to produce a slightly enhanced virulence. Infection was produced much more readily by injecting 24- to 48-hour broth cultures than by using saline emulsions of glucose agar slopes.

Saline emulsions of old sporing cultures upon inspissated serum produced only a transitory swelling and a little area of crepitation; the guinea-pigs showed no sign of depression or malaise. It should be observed that the toxin produced by *B. perfringens* is so feeble that it takes 10 to 12 c.c. of a filtered broth culture to kill a guinea-pig in twelve to eighteen hours.

In two strains it was found that 60 to 100 million organisms from a 24- to 48-hour broth culture, diluted with saline, was a lethal dose for guinea-pigs of about 250 to 300 grms. The guinea-pigs displayed, however, a considerable variation in susceptibility, and over a series of fifty-six pigs, inoculated with a lethal dose, 12.5 per cent. recovered spontaneously after severe illness. A few hours after the injection of a lethal dose an extensive cedema may already be present with a certain amount of gas, the cedema spreads, and the guinea-pig may die in from twelve to eighteen hours.
At the post-mortem examination the subcutaneous tissue shows a very large amount of transparent blood-stained oedema, which may be gelatinous, especially in a rapid case. The peritoneum is injected, lungs and liver are normal in appearance, the suprarenal bodies are as a rule coloured deep red, if death has taken place within twenty-four hours of inoculation. This sign is absent if the animal survives until the third day. The tissues at the site of inoculation, e.g., the muscles of the thigh, are friable and pale in colour on the outer surfaces, while the inner parts may be coloured a curious deep red. In a late case this destroyed condition of the tissue may be found in any part of the body where there is a considerable muscle mass, very frequently, for instance, in the opposite limb to the one inoculated. There is a characteristic acid smell but no odour of putrefaction, and I have never seen any blackening of the tissues. In early cases, where death supervenes within twenty-four hours, gas is found in the tissues, but less than in a similar case of Rauschbrand infection. In a guinea-pig which dies about the end of the third or fourth day there is very much less gas in the tissues than in the earlier cases.

The organism can be found in large numbers in the oedematous fluid in any part of the body, in the tissues of the liver, and can usually be recovered from the heart's blood of an animal killed in extremis.

In instances where the pig shows a large oedematous swelling, but is able to survive for some days, the condition frequently culminates in a wet perforating gangrene of the limb and abdomen. These gangrenous cases not infrequently recover, and even where there has been as much as 2 or 3 square inches of destroyed epidermis, the healing process is so complete that very little if any sign is left after three or four weeks. If the gangrene has occurred in the limbs, there may sometimes be a retracted cicatrix. Sometimes a guinea-pig will recover from a lethal dose after the production of an oedema which stretches from the limb inoculated right over the whole abdomen to the axillary region on both sides, without there having been any perforating gangrene to relieve the condition; this is, however, rare. An infecting but sublethal dose will very generally produce a local gangrene.

(2) Bacillus of the Malignant Edema Type (Koch).—This organism is motile and Gram-positive, but Gram-negative individuals are frequent in older cultures. Flagella can be demonstrated by staining appropriately. Surface colonies upon glucose agar are characterised by the tangle of filaments which grow out from the centre and give rise to a curious woolly appearance; the filaments grow down into the medium, and only a small part of the colony can be lifted by touching the surface with a needle. In deep plates and shakes the colonies are likewise woolly. When grown on inspissated serum there is an active
digestion, and the medium is liquefied in the course of a few days; the cooked meat is digested and blackened with the production of a penetrating odour of putrefaction. (This blackening is a useful reaction, and it should be noted that if the meat medium happens to be acid instead of slightly alkaline, the blackening may be delayed for a few days or may even not occur at all; the anaerobes in general do better in this meat medium if the reaction is alkaline.)

Milk is rapidly digested without the formation of a clot, though there is generally a certain amount of precipitation of the casein in grains, which are ultimately digested. The reaction in all these media is alkaline after a few days' growth. Gelatin is liquefied. This type of organism does not ferment glycerin, inulin, or starch, but is able to ferment glucose to a certain extent. The organism forms spores readily in all the media; they are central, subterminal, or even terminal.

This malignant oedema bacillus was found to be only slightly pathogenic for guinea-pigs in pure culture; this was equally true of the strains isolated by myself from the wound material and of the various laboratory strains tested. Thus it required from 5 to 8 c.c. of a 48-hour broth culture to produce a perforating gangrene, and on no occasion was death produced by a pure broth culture of malignant oedema.

Injection of malignant oedema mixed with *B. perfringens* produced very serious results; the former bacillus was always capable of development in these cases. An extensive oedematous swelling of the limb inoculated and of the abdomen is generally produced in a few hours; the skin takes on a shiny blistered appearance, and the hair may come off; death may supervene at this stage. If the guinea-pig survives for twenty-four hours, the skin usually becomes wet and greenish at the site of the oedema, and then rapidly breaks down and becomes gangrenous. There is a very characteristic fetid odour from an infection of this kind. The animal may die after the gangrene has been produced, 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. perfringens* and malignant oedema, 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 which is stained red and which presents an oily appearance. The oedema extends as a rule over the whole abdomen up to the axillary region. The limb inoculated is also oedematous, and the tissues are friable in consistency and may be dark in colour. There is a little but not very much gas; the odour is very fetid and quite distinct from that produced by a pure *perfringens* oedema. The peritoneum is injected and purple in colour; lungs, liver, and spleen are normal; hemorrhagic patches are sometimes to be observed in the subcutaneous tissues.
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Both organisms are found to be present in large numbers in the exudate and the tissues; they are also found in the liver and the spleen. The surface of the liver shows the long filamentous forms of the malignant oedema bacillus so often noted in the literature. The malignant oedema bacillus is actively motile in the animal body, and spore-bearing rods may be found as early as twenty-four hours from the time of inoculation, though they are very much more frequent a little later on.

(3) Hibler IX.—This bacillus coincides with the form which von Hibler calls No. IX (1908 11). The points of divergence are quite unimportant; they are, firstly, that the form isolated by myself is even less often motile and more generally non-motile than von Hibler's strain. And, further, the spore in von Hibler's strain is described as being slightly rounder in shape than in those obtained by myself. As I have said above, it is highly probable that the organism in question is the same as Rodella III, but the author gives an insufficiently detailed account of his bacillus.

The organism isolated by myself and entitled H IX has a very characteristic appearance in the sporing condition, the bacillary rod is relatively rather long and very slender and not infrequently curved. The fully formed spore is oval, though the difference between the long and the short axis is in many cases not very great; it adheres to the rod even after its development appears to be complete. Spores are occasionally formed at either end of the rod. The organism is Gram-positive, but it has a strong tendency to be Gram-negative, especially in broth cultures. The surface colonies on glucose agar are flat and usually crenated at the edges or slightly irregular in shape, but filamentous outgrowths are not formed. Older colonies may show a somewhat striated or watered milk appearance; they do not grow down into the medium. There is a marked tendency for the organism to grow over the plate in a more or less uniform surface film; it is advisable to use rather dry plates. In deep shakes the colonies are lenticular or may grow out into rather irregularly shaped lumps.

The organism does not liquefy gelatin or inspissated serum; upon the meat medium it develops a considerable quantity of gas, but does not cause any blackening. It attacks milk very slowly, but does as a rule produce a soft clot in about eight to ten days; the growth is not very prolific in milk. There is no growth upon the undiluted alkaline egg fluid; the growth is rather poor upon ordinary broth, in which medium the bacillus has a marked tendency to be Gram-negative; it also does not form spores readily under these conditions, so that broth cultures have a curiously aberrant appearance. The organism produces both acid and gas in glucose; these cultures resemble those upon ordinary broth in respect to the morphology of the bacillus; the growth is, however, much more prolific. Inulin and glycerin are not fermented.
There is a certain definite capacity for fermenting starch, but this varies a little with the vigour of the material inoculated, and is never as pronounced as that shown in regard to glucose. The organism is non-pathogenic for guinea-pigs, and there is no evidence of the production of a toxin.

As H IX develops large quantities of gas, when grown in the meat medium, its presence in an active state in a wound would probably make a certain contribution to a gas-gangrene condition. The form is further important owing to its morphological resemblance with B. tetani, though the rods of the latter are rather stouter and the spores rounder, also clostridial forms are much more frequent in a tetanus culture. As will be seen from Table II., H IX turns up mixed with the malignant edema bacillus in the wound material; such a mixed culture gives a very tetanus-like picture, as there are motile rods present, and the protein media are digested, and there are large numbers of endsporing drumstick individuals. If tetanus were eliminated by an animal test, such a mixture would present an appearance not unlike that described for B. putrificus cultures.

Table I. gives the cultural reactions of these three types isolated from wounds, and also of a Vibrion septique strain, a laboratory strain of malignant edema, and a tetanus culture.

So much then for the actual types which were finally obtained in pure culture; it is of interest now to touch upon the question of their isolation and the combinations in which they turned up.

Table II. gives the incidence of the forms and the findings in the individual cases. In drawing up the table I have considered the presence of a given bacterial type to be "demonstrated" when its growth reactions and characters were clearly determined in the culture isolated, but, for reasons to appear below, I do not reckon these to be pure cultures unless they have been exposed to rigorous cultural tests, and repeated platings carried out over a long period. Where the strains have been treated in this way and proper assurance of the purity obtained, they are put down as "isolated in pure culture."

Where the presence of an organism was noted upon any lesser data than a clear demonstration of all its characters, it is put down as "indicated."

The results given in Table II. may be considered as stating the positive findings. On the other hand, in working with the anaerobes, one is left with the conclusion that the negative deductions from such a collection of data are of relatively little value. It is generally pretty easy to get cultural evidence of anaerobic organisms in the material taken from cases of wound gangrene, but it is quite another matter to get all the anaerobes originally present to survive in anything like pure culture. Over and over again it was found that where at the start there had been evidence of one or more anaerobic species in the material, one or other (and occasionally all) would die
<table>
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<th>Organism</th>
<th>Motility</th>
<th>Spores</th>
<th>Colony on Agar</th>
<th>Reaction in Meat</th>
<th>Milk</th>
<th>Gelatin</th>
<th>Impulsated Serum</th>
<th>Glucose Broth</th>
<th>Inulin</th>
<th>Glycerin</th>
<th>Starch</th>
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<td>*Vibrio septi-*que (Pasteur Institute)</td>
<td>Motile, but very often non-motile.</td>
<td>Central or sub-terminal.</td>
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<td>Acid reaction, gas, pink colour.</td>
<td>Acid clot, some gas, no digestion.</td>
<td>Liquefied</td>
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<td><em>B. perfringens isohbed</em> from wounds, 1914-15</td>
<td>Non-motile.</td>
<td>Central or sub-terminal.</td>
<td>Smooth surface colony, lentil-</td>
<td>Acid reaction, much gas, pink colour.</td>
<td>Acid clot, much gas, no digestion.</td>
<td>Liquefied</td>
<td>Not liquefied</td>
<td>Acid and gas</td>
<td>Acid and gas, but variable.</td>
<td>Acid and gas, but vari-</td>
<td>No change in medium.</td>
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<td>H IX, isolated from wounds, 1914-15</td>
<td>Non-motile or only very feebly motile.</td>
<td>Endospore attached to long slender rod.</td>
<td>Flat, slightly crenated smooth colony on surface, has lumpy outgrowths in depth.</td>
<td>Acid reaction, considerable amount of gas, pink colour.</td>
<td>Attacked very slowly, finally acid and clot.</td>
<td>Not liquefied</td>
<td>Not liquefied</td>
<td>Acid and gas</td>
<td>No change.</td>
<td>No change.</td>
<td>Acid and gas, but varies a little.</td>
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<td>Malignant <em>odema bacillus</em> (Koch); isolated from wounds, 1914-15</td>
<td>Actively motile.</td>
<td>Central or sub-terminal, rarely terminal.</td>
<td>Wooly colony, both on surface and in depth.</td>
<td>Alkaline reaction, blackened with putrid odour, digested.</td>
<td>Milk digested without clot, but casein may be precipitated in grain.</td>
<td>Liquefied</td>
<td>Liquefied</td>
<td>Acid and gas, but not very vigorous.</td>
<td>No change.</td>
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<td>Malignant <em>odema</em>, laboratory strain (Dr. O'Brien).</td>
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<td>Tetanus culture, from Dr. O'Brien.</td>
<td>Motile.</td>
<td>Endospore, drumstick, but clostridial forms present.</td>
<td>Wooly colony.</td>
<td>Blackened with putrid odour.</td>
<td>Digested, with precipitation of grains of casein, which are digested.</td>
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<td>An anaerobe with smooth colony, Gram-positive large rod with slightly rounded end, no spores; did not grow in subcultures, possibly \textit{B. perfringens}.</td>
<td>Malignant oedema (Koch), isolated in pure culture.</td>
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<td>(B. perfringens; malignant oedema (Koch). )</td>
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<td>(B. perfringens, malignant oedema; both isolated in pure culture. )</td>
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<td>(B. perfringens, isolated in pure culture; malignant oedema demonstrated. )</td>
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<td>10</td>
<td>An anaerobe forming gas in deep glucose shake, smooth colony; Gram-positive rod, failed to grow from deep glucose agar culture sent. (?) \textit{B. perfringens}.</td>
<td>(B. perfringens, isolated in pure culture; malignant oedema demonstrated. )</td>
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<td>(B. perfringens; malignant oedema )</td>
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<td>12</td>
<td>An anaerobic organism which failed to grow.</td>
<td>Malignant oedema and Hix.</td>
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<td>P 1</td>
<td>An anaerobe with smooth colony and forming spores, very shy grower, not enough data for diagnosis.</td>
<td>(B. perfringens, isolated in pure culture; Hix; malignant oedema. )</td>
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<td>2</td>
<td></td>
<td>(Hix, isolated in pure culture; malignant oedema. )</td>
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<td>3</td>
<td>An anaerobe with smooth colony which clotted milk, with production of gas, Gram-positive stout rod, failed to grow it out, possibly \textit{B. perfringens}.</td>
<td>Malignant oedema.</td>
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<td>4</td>
<td></td>
<td>(Hix, isolated in pure culture. )</td>
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<td>5</td>
<td></td>
<td>Malignant oedema.</td>
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<td>6</td>
<td>An anaerobe with smooth colony, failed to get it to grow out.</td>
<td>(B. perfringens \textit{in pure culture; malignant oedema.} )</td>
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<td>7</td>
<td></td>
<td>(B. perfringens \textit{in pure culture; malignant oedema.} )</td>
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<td>8</td>
<td>An anaerobic organism which failed to grow.</td>
<td>Malignant oedema.</td>
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<td>9</td>
<td></td>
<td>(Hix, isolated in pure culture. )</td>
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<td>10</td>
<td></td>
<td>Malignant oedema.</td>
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<td>11</td>
<td></td>
<td>(Hix, isolated in pure culture. )</td>
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<td>12</td>
<td>An anaerobe with smooth colony, stout Gram-positive rod; failed to recover it.</td>
<td>(B. perfringens \textit{in pure culture; malignant oedema.} )</td>
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<td>13</td>
<td></td>
<td>(B. perfringens \textit{in pure culture; malignant oedema.} )</td>
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<td>14</td>
<td></td>
<td>(B. perfringens \textit{in pure culture; malignant oedema.} )</td>
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K 1, infected hemothorax.
K 2, infected hemothorax.
K 3, infected hemothorax.
K 4, heart blood, generalised gas gangrene.

An anaerobic organism which failed to grow.
An anaerobic organisms which failed to grow.
An anaerobic organisms which failed to grow.
TABLE II.—continued.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>K 5, generalised gas gangrene material from liver.</td>
<td>A slender endsporing organism which escaped isolation.</td>
<td>B. perfringens isolated in pure culture.</td>
</tr>
<tr>
<td>K 6, infected hemothorax generalised gas gangrene.</td>
<td>No anaerobes were obtained from the material sent.</td>
<td>Malignant oedema; B. perfringens, isolated in pure culture.</td>
</tr>
<tr>
<td>K 8, infected hemothorax.</td>
<td>An anaerobe with a smooth colony which escaped isolation.</td>
<td></td>
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<tr>
<td>K 9, generalised gas gangrene.</td>
<td>Anaerobe with woolly colony, not isolated.</td>
<td>B. perfringens, isolated in pure culture.</td>
</tr>
<tr>
<td>K 10, infected hemothorax.</td>
<td>Several anaerobes still under consideration, one of which is apparently malignant oedema.</td>
<td></td>
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<tr>
<td>K 11</td>
<td></td>
<td></td>
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<tr>
<td>K 12</td>
<td></td>
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<tr>
<td>K 13</td>
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</tbody>
</table>

out before it was isolated in pure culture. My own experience is that the perfringens group and the non-liquefiers of gelatin were more easily lost in the handling than the malignant oedema type, nevertheless in some few cases this bacillus did apparently not survive.

In general, B. perfringens, which was very frequently present, will outgrow any other anaerobe in a milk- or sugar-containing medium during the first twenty-four to forty-eight hours. In milk the acid presently inhibits the further growth of B. perfringens and also prevents such an organism as malignant oedema from making more than a very feeble growth, which is not sufficient to produce any observable effect upon the medium. Thus a mixed culture of B. perfringens and malignant oedema in milk will almost always give the characters of a perfringens culture in regard to acid, gas, and clot, unless the malignant oedema is present in quite overwhelming numbers. Malignant oedema appears to make little headway in milk in the presence of B. perfringens, but in many cases it could survive through several passages in the milk medium, although the general character of a pure perfringens culture such as acid reaction, clot, and gas were maintained.

If, however, such a mixed culture, in which the malignant oedema bacillus is unsuspected, is inoculated into the cooked meat medium, or alkaline egg fluid, the course of events is generally as follows: during the first few days B. perfringens is again the obvious organism; later, however, sometimes after six to ten days, but more often only after as much as fifteen to thirty days of anaerobic incubation, the malignant oedema organism obtains the upper hand, the culture gives off a putrid odour, and the form found to be in possession is a motile sporulating
bacillus. Upon plating out there may appear both smooth and woolly colonies, or only the latter. That is to say, that in milk or a medium rich in fermentable sugar, _B. perfringens_ may persistently mask the presence of the proteolytic type, without, however, as a general rule, causing it to die out entirely; in an alkaline or neutral medium rich in protein the malignant oedema organism will, if anaerobic incubation is continued, overcome _B. perfringens_, and the general growth characters of a malignant oedema culture will be shown.

The literature suggests that workers are inclined to allow an extraordinary latitude in the matter of motility, spore formation, character of colony, and cultural reactions to organisms of the anaerobic group; and this, moreover, in strains under observation during relatively long periods of time. Thus Klein's _B. enteritidis sporogenes_ is permitted at one time to appear as a non-motile carbohydrate fermenter, and at another as a motile protein digester. But it is interesting to note that this variability disappeared when Klein's original strain was handled by von Hibler. This worker isolated two organisms from Klein's strain, namely, _B. cadaveris sporogenes_, a proteolytic form, and _B. enteritidis sporogenes_, which was a butyric acid bacillus. Von Hibler describes it as motile, but as quite constant in its characters. (See Table III. for reactions.)

Such careful workers as Grassberger and Shattenfroh (1908) give forth statements of the following nature in respect to Rauschbrand strains which they had under observation for years.

"It has become clear that in every single strain of these non-motile bacteria, including the bacilli of Fraenkel, upon passage on to inspissated serum or on to heated egg medium, the capacity of sporulation and a pronounced motility are once more called forth." And again, "One is concerned, therefore, with the following characteristics, to summarise in brief the peculiarities of the butyric acid bacteria: to type 3 (the Rauschbrand type) belong a number of bacteria which oscillate between butyric fermentation and an energetic capacity for attacking proteins."

Fraenkel (1893), who seems to have had a bacillus identical with _B. perfringens_, mentions, however, that inspissated serum was liquefied with the production of a markedly putrid odour; this does not occur in a really pure culture of the gas phlegmon or _perfringens_ bacillus.

The instances which I have just quoted are by no means isolated examples in the literature, and I have drawn attention to them to show that either we must consider the sporing anaerobes as extraordinarily polymorphic, or we must conclude that there are some special difficulties in obtaining pure cultures and that certain of the anaerobes appear to be well adapted to live in very close association with each other.

The results of this investigation of the wound anaerobes lead me to consider that mixed cultures account for most of the anomalies
CERTAIN ANAEROBES ISOLATED FROM WOUNDS. 339

cited. And this cause of error must be rigorously excluded before a mutation or a variation under cultivation theory is advanced as an explanation of such findings as those of Grassberger and Shattenfroh. It appears to me that any such hypothesis must be preceded by a critical inquiry into the value of the methods of isolation.

My own experience has been that with one single exception all the perfringens strains isolated from gangrenous wound were found to contain some other anaerobe, most frequently a proteolytic organism of the malignant oedema type, which in certain cases did not make itself manifest until the strain was cultivated upon cooked meat, alkaline egg fluid, or inspissated serum (though this last medium is sometimes not so good for this purpose as the other two), and incubation under anaerobic conditions kept up for a period exceeding twenty to thirty days. Thus three cultures (SR 8, SR 9, and SR 12) yielded obvious perfringens cultures, and malignant oedema was only found to grow out after a relatively long period of cultivation.

In other cases both the organisms were manifest from the first, and after B. perfringens was plated out and appeared to be isolated in pure culture, malignant oedema was still found to be present. This difficulty in freeing the perfringens culture from the malignant oedema types persisted through a number of platings, so that at one period in the research my results appeared to resemble those obtained by Grassberger and Shattenfroh, in so far as a non-motile butyric acid producing bacillus gave rise upon occasion to a spore-bearing motile proteolytic bacillus. Finally, I isolated single bacilli from what I took to be a pure culture of B. perfringens, and after a very large number of failures got one which grew. This single bacillus strain (K 13 I B) was cultivated upon all the media, and over seven months' observation has never shown any appreciable variation in its characters. It behaves with as little attempt at drastic mutation as any other well-defined species of bacterial organism. This encouraged me to persevere with the work of obtaining really clean cultures of the other strains of perfringens derived from wounds, and after quite a disproportionate amount of labour and repeated platings, following directly upon each other without intervening cultivation, I did ultimately get six strains, in addition to the single bacillus subcultures, which no longer gave any motile proteolytic forms, even under conditions most favourable to their production. I wish to lay especial stress upon the fact that in several of these cases the presence of a proteolytic type was quite unsuspected, through a number of platings upon glucose agar, and some varying number of weeks of cultivation upon the various media (exclusive, however, of the egg fluid), it being my practice at the time to grow the cultures in an anaerobic cylinder for three to five days, and thereafter to leave them in the air at room temperature; they were not sealed with paraffin.

It should be noted that upon plates which showed only smooth
discrete colonies of the smooth *perfringens* type, it was found, upon emulsifying a number of colonies in saline and examining on a warm stage, that motile rods could be detected in a few instances. These mixed colonies were impossible to detect in stained preparations.

In the earliest days of this investigation the method, including as it did drastic heating after enriching, favoured the growth of malignant oedema to the final exclusion of everything else, and though smooth colonies are recorded, composed of stout Gram-positive rods and showing no spores, they could not be got to grow on the media then used, and one is not in a position to assert or deny the presence of *B. perfringens*, although from subsequent experience one is not left in much doubt that the smooth colonies were *B. perfringens*. The bacteriological findings in the first nine cases thus work out, probably owing to the method adopted, with the proteolytic organism of the malignant oedema type as the sole surviving anaerobe.

*H* IX was obtained not infrequently, and appeared in combination with both *B. perfringens* and with the malignant oedema type. The difficulties of disentangling malignant oedema from *H* IX were quite as great as in the case of *perfringens* and malignant oedema. Colonies picked off and replated several times in succession, giving clean-looking plates, and then grown in the various media, would for weeks show as pure *H* IX cultures, and would nevertheless, in the long run, show the malignant oedema type upon the favourable media after subculture and long incubation. In many cases, where the malignant oedema could be detected, it was present in very small numbers, and upon plating it did not grow up in colonies of its own, but only came up in little filamentous patches actually within the colonies of the *H* IX. And, moreover, a further difficulty was present in that even these little patches did not appear in many cases until the plate had been incubated in the anaerobic cylinder for more than four or five days.

A most disconcerting feature is that in certain of these mixed cultures the two organisms seem to arrive at a sort of symbiotic balance and behave in a very uniform way for long periods of time, so that one is persuaded, upon the cultural evidence, that one is dealing with a cultural entity. Thus one mixture of *H* IX and malignant oedema gave a uniform set of cultural reactions for some months which were different from any I had hitherto found. They could, however, in the long run be disentangled into two pure strains.

In general, then, it may be urged that where work of any greater scientific moment than mere diagnosis is required, the most scrupulous care in testing the purity of the cultures is necessary; the ordinary precautions of a small number of platings and a uniform set of subcultural reactions over a few weeks is quite inadequate. The final criterion is, of course, the single bacillus method, though with the anaerobes this is, it must be confessed, a counsel of perfection. One
would particularly urge that this scrupulous care be taken with the
cultures, used as the basis of serological work. The confusion in the
literature in regard to cultural reactions due to mixed cultures is
already sufficiently baffling, and it is obvious that no help will come
from the serological side unless especial care is taken to start from a
clean basis. In the material sent to me it will be seen that the
proteolytic organism, which I have, for reasons to be discussed in the
next section, considered to belong to the malignant oedema type,
appeared very frequently. From papers written on the subject since
the outbreak of the war there seems to be a tendency to neglect this
organism. There is no doubt that B. *perfringens* does by itself give
rise to a very serious condition. I would like, nevertheless, to suggest
that the additional presence of the malignant oedema bacillus of Koch
is a circumstance worthy perhaps of greater attention than it is at
present receiving.

Having described the anaerobic organisms found in the material
from a certain group of gangrenous wounds, it remains to discuss the
justification of the nomenclature applied.

In dealing with the literature concerned with the pathogenic
anaerobes, one is confronted with many organisms named but incom-
pletely described, with many obscurities and with a considerable
amount of controversy. The confusions have arisen partly owing to
the difficulties of obtaining pure cultures, and partly owing to the
absence of any attempt at systematising the characters and reactions
upon which the diagnoses are to be made. A very considerable
amount of order has, however, been brought into the chaos by the work
of von Hibler, "Die pathogenen Anaeroben" (1908). Von Hibler's
book is insufficiently indexed, not very well arranged, and difficult to
read. As this work, however, does give a basis for a rational classifi-
cation of the spore-bearing anaerobes into well-defined groups upon a
definite series of characters, I have tabulated his more important
results so as to render them capable of rapid apprehension and com-
parison with those obtained by other workers.

I have selected from von Hibler's book the results of his researches
upon the growth reactions of the more important types upon milk,
inspissated serum gelatin, and "Hirnbrei." I have myself made use
of this last medium also, and find that perfectly comparable reactions
are obtained by substituting the cooked meat medium mentioned above.
In considering, therefore, the data in the table compiled from von
Hibler in relation with my own results, the "Hirnbrei" reaction can
be compared quite strictly with that of the cooked meat.

Table III. is put together directly from the data given in von
Hibler's book. The obvious criticism of his work is that there
scarcely seems to be sufficient authority for retaining so large a number
of species. I have purposely omitted his results upon the heat
resistance of the spores, to which he attaches considerable diagnostic
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<tbody>
<tr>
<td>III. Bacillus of Ghou and Sachs (B. des méthix d'ardeau).</td>
<td>+</td>
<td>Spores usually central.</td>
<td>Surface colony smooth; deep colony has filamentous processes.</td>
<td>&quot;Citron&quot; and &quot;bladder&quot; forms occur; long threads are formed on surface of liver, etc.; forms spores readily in animal body.</td>
<td>&quot;</td>
<td>Acid reaction; does not blacken.</td>
<td>Acid, clot; some gas.</td>
<td>Medium is liquefied.</td>
<td>Medium not liquefied.</td>
</tr>
<tr>
<td>V. Bacillus of Novy (B. actinotus malignum).</td>
<td>Motile, but condition variable.</td>
<td>Spores usually central; alkaline reaction favourable.</td>
<td>Surface colony smooth; deep colony has filamentous processes.</td>
<td>&quot;Citron&quot; and &quot;bladder&quot; forms occur, but not frequent; does not grow in long threads; does not form spores readily in animal body.</td>
<td>&quot;</td>
<td>Acid reaction; does not blacken.</td>
<td>Acid, clot; little gas.</td>
<td>Medium is liquefied.</td>
<td>Not liquefied.</td>
</tr>
<tr>
<td>II. B. phlegmones capheicatetor, Fraenkel (B. perfringens).</td>
<td>-</td>
<td>Spores central; forms spores in alkaline media, rich in protein.</td>
<td>Surface colony lenticular or whetstone shaped.</td>
<td>Shows capsule; does not occur in long threads.</td>
<td>&quot;</td>
<td>Acid reaction; does not blacken; much gas.</td>
<td>Acid, clot; develops much gas.</td>
<td>Medium is liquefied.</td>
<td>Not liquefied.</td>
</tr>
<tr>
<td>IV. B. enteritidis ssp. sporogenes (Klein).</td>
<td>+</td>
<td>Spores usually central.</td>
<td>Smooth surface colony; deep colony lenticular.</td>
<td>&quot;Citron&quot; and &quot;bladder&quot; forms frequent; does not form long gas.</td>
<td>&quot;</td>
<td>Acid reaction; does not blacken.</td>
<td>Acid, clot; develops much gas.</td>
<td>Medium is liquefied.</td>
<td>&quot;</td>
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Table III.


<table>
<thead>
<tr>
<th>Organism</th>
<th>Motility</th>
<th>Spores</th>
<th>Colony on Agar</th>
<th>Growth in Animal Body</th>
<th>Pathogenicity</th>
<th>Reaction in &quot;Himbrey's&quot;</th>
<th>Reaction in Milk</th>
<th>Reaction in Gelatin</th>
<th>Reaction on Implanted Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. vin.</td>
<td>Motile, but condition variable.</td>
<td>Spores usually central; alkaline medium favourable.</td>
<td>Smooth surface colony; deep colony has filaments.</td>
<td>..</td>
<td>Non-pathogenic.</td>
<td>Acid reaction does not blacken.</td>
<td>Acid, clot, very slowly.</td>
<td>Medium not liquefied.</td>
<td>Not liquefied.</td>
</tr>
<tr>
<td>VI. B. angio-bacter.</td>
<td>Motile, but varies.</td>
<td>Spores usually central.</td>
<td>Smooth surface colony; deep colony has filaments.</td>
<td>..</td>
<td>&quot;&quot;</td>
<td>Acid reaction does not blacken.</td>
<td>Acid, clot; much gas.</td>
<td>Medium not liquefied.</td>
<td>&quot;&quot;</td>
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<tr>
<td>H. us (derived from emphysema of arm following fracture).</td>
<td>Motile, but very often non-motile.</td>
<td>Spores typically terminal; acid reaction inhibits formation of spores.</td>
<td>Smooth surface colony; deep colony has irregular outgrowths.</td>
<td>..</td>
<td>Non-pathogenic experimentally.</td>
<td>Acid reaction does not blacken.</td>
<td>Attacks milk very slowly; finally acid, and slight amount of clotting.</td>
<td>Medium not liquefied.</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>X. B. adenatitis maligni (Koch).</td>
<td>+</td>
<td>Subterminal, central, or terminal; oval in shape.</td>
<td>Surface and deep colonies are woody.</td>
<td>&quot;Citron&quot; and &quot;bladder&quot; forms very rare; grows in long threads on surface of liver.</td>
<td>Pathogenic.</td>
<td>Alkaline reaction; putrid odour, blackened, digested.</td>
<td>Digested; casein precipitated at first in grains; reaction finally alkaline.</td>
<td>Liquefied.</td>
<td>Liquefied.</td>
</tr>
<tr>
<td>XII. B. tetani (Nicolai).</td>
<td>+</td>
<td>Endospores, round in shape.</td>
<td>Surface and deep colonies are woody.</td>
<td>Does not show &quot;granulose&quot; or &quot;bladder&quot; forms.</td>
<td>Pathogenic.</td>
<td>Alkaline reaction; putrid odour, blackened.</td>
<td>Digested; casein precipitated at first in grains; reaction alkaline.</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>XIII. B. botulinus (Van Krusegem).</td>
<td>+</td>
<td>Endospores, but may be central.</td>
<td>Surface and deep colonies are woody.</td>
<td>Pathogenic, but seldom produces death in experimental animals.</td>
<td>Alkaline reaction; putrid odour, blackened.</td>
<td>Digested; casein precipitated at first in grains; reaction alkaline.</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
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</tr>
<tr>
<td>XIV. B. cadaveris sporogenes (Klein). B. putrificus (Bienstock).</td>
<td>+</td>
<td>Endospores, round in shape.</td>
<td>Surface and deep colonies are woody.</td>
<td>Not markedly pathogenic.</td>
<td>Alkaline reaction; putrid odour, blackened.</td>
<td>Digested; casein precipitated in grains; reaction alkaline.</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
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</table>
importance. The method adopted in this part of his research has so many sources of error that it is impossible to place much reliance upon his results in detail. Von Hibler used cultures upon various media, and inoculated samples into the bottom of "Hirnbrei" tubes with a pipette; these he exposed to streaming steam for varying periods of time. The tubes were then incubated for some days, and he took the absence of growth as evidence that all the spores had been killed by exposure to a certain temperature for a certain number of minutes. To consider only one source of error, he had no means of ascertaining the exact reaction of the fluid surrounding the spores. The great importance of the influence of acid upon the death-rate of bacteria exposed to heat has been shown by Miss Chick (1910). She found that 0.5 N acetic acid added to 20 c.c. of broth culture of \textit{B. typhosus} increased the death-rate of the organism exposed to 54° C. more than forty times, when compared with the death-rate of the organisms in the same broth at the same temperature without the addition of the acid. The effect of the reaction on the death-rate of spores may be different, but it can obviously not be neglected. In addition, he did not count the number of spores introduced, which is a serious omission in view of the fact that he was ascertaining an end-point and not a death-rate. Von Hibler's results in this direction may prove upon repetition, under more careful conditions, to have a diagnostic significance, but for the moment they cannot be looked upon as sufficiently established.

It is, however, of no great importance whether all the species admitted by von Hibler can be upheld or not; the serious merit of his work is that in spite of occasional obscurities and contradictions he has furnished the particulars of a number of well-studied strains subjected to a uniform series of tests. Moreover, he seems to have been alive to the necessity of taking quite special pains before assuming the purity of the cultures employed.

Below is a table of von Hibler's results, using his names and numbers for the types included.

I have grouped the forms according to their reactions; this arrangement is not taken from von Hibler.

The order of the table is as follows:

<table>
<thead>
<tr>
<th>I. Bacillus of Rauschbrand</th>
<th>A. Rauschbrand group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>III. Bacillus of Ghon and Sachs</td>
<td>B. Perfringens group.</td>
</tr>
<tr>
<td>V. Bacillus of Novy</td>
<td>C. Non-liquefiers of gelatin group.</td>
</tr>
<tr>
<td>II. \textit{B. phlegmones emphysematose} (\textit{B. perfringens})</td>
<td>D. Proteolytic group.</td>
</tr>
<tr>
<td>IV. \textit{B. enteritidis sporogenes} von Hibler vii</td>
<td></td>
</tr>
<tr>
<td>VIII. \textit{B. amylobacter} von Hibler ix</td>
<td></td>
</tr>
<tr>
<td>X. \textit{B. edemaasis maligni} (Koch)</td>
<td></td>
</tr>
<tr>
<td>XII. \textit{B. tetani}</td>
<td></td>
</tr>
<tr>
<td>XIII. \textit{B. botulinus}</td>
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<tr>
<td>XIV. \textit{B. cadaveris sporogenes}</td>
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CERTAIN ANAEROBES ISOLATED FROM WOUNDS.

From the characters shown in this table one can arrange the organisms in question into the four groups mentioned above.

**Group A**, which may be called the Rauschbrand group, containing forms which do not digest the proteins; they do not liquefy inspissated serum or produce blackening in the meat or "Hirnbrei" medium. They clot milk without much shrinkage of the clot, they are able to liquefy gelatin, they are usually motile, but this character is liable to variation. Spores are formed in all the media, but an alkaline reaction favours their production—the spores are central or subterminal. The group is for the most part very pathogenic for the laboratory animals. They are very strict anaerobes.

**Group B**, which is the *perfringens* group, contains forms which have very little capacity for attacking the proteins, though, as was pointed out by Tissier and Martelly (1902) in their excellent work on the "Putréfaction de la Viande de bouchérie," they are able to a certain slight extent to peptonise milk. The strains belonging to the *perfringens* group do not liquefy inspissated serum or blacken meat or "Hirnbrei"; they clot milk, producing a hard, tough clot much broken by gas; the clot shrinks, leaving a clear whey; the reaction is acid, and a strong odour of butyric acid is given off. There is no obvious digestion of the clot. The *B. perfringens* group are non-motile organisms with the exception of *B. enteritidis* sporogenes of Klein, which seems to be an ill-defined species. The colonies are smooth, spores are not formed except upon alkaline media, rich in protein, and containing little fermentable sugar. The organisms are pathogenic.

**Group C** may be recognised by their inability to liquefy gelatin; they are incapable of attacking the proteins, and do not liquefy inspissated serum or blacken the meat media.

Some question may be raised as to the propriety of the form, marked VIII. *B. amylolyticus*, being placed in this group. I have included it in this place because of its behaviour upon gelatin. Von Hibler's strains vii and ix it will be observed, attack milk very slowly, forming a soft clot after a long period, whereas the amylolyticus bacillus forms a tough clot, broken by gas, with the production of acid.

**Group D** may be called the proteolytic group and is characterised primarily by its very active powers of digesting media rich in protein. Milk is digested usually without the production of clot, though a precipitation of casein in grains may occur; inspissated serum is liquefied, meat media blackened and digested, and gelatin is liquefied; their growth produces a penetrating odour of putrefaction. The members of this group are actively motile; the colonies have a characteristic appearance, and grow out into long tangled filaments at their edges. Such members as *B. tetani* and *B. botulinus* are sharply marked off by their characteristic toxins and other peculiarities, but they are none the less closely allied to the rest of the group in their cultural reactions.
This division of the pathogenic anaerobes into groups, according to their reactions in a uniform set of culture media, affords a useful starting-point for the study of the particular forms met with in any investigation involving this type of organism. I attach importance to the groups, as at the present stage of our knowledge the species may perhaps be further subdivided in accordance with the development of more delicate tests.

It will be seen that the strains isolated from the wound material, which I have called *B. perfringens*, fit into Group B (*perfringens* group), and are perfectly typical members; there is no point of difference, and they may bear the name assigned to them without further argument.

In like manner, the wound strains classed by me as H IX belong clearly to Group C (the non-liquefiers of gelatin), and coincide with the particular representative of the group designated in von Hibler's book by the number IX. It is interesting to note in passing that von Hibler isolated his strain from an emphysematous condition in the arm of a boy, subsequent upon a bad fracture, and the arm had finally to be amputated.

The question of the identification of the wound strains belonging to the proteolytic group with the bacillus of malignant *œdema* is clearly a more debatable point. The organism isolated by myself will at once be allowed its place in Group D (the proteolytic group), but the reasons for considering that it may be designated as *B. adenatis maligni* (Koch) must be gone into more fully. It will be seen that the proteolytic wound strains which I obtain agree in the cultural reactions, in the character of the colony and in morphology, with the malignant *œdema* bacillus of Koch as described by von Hibler (see Table III.), and that they do not agree with the malignant *œdema* bacillus of Novy, or with the malignant *œdema* bacillus of Ghon and Sachs (1904 10). Von Hibler, it should be noted, worked with Ghon and Sachs' actual strain.

It is impossible within the range of the present paper to go into the very vexed question as to what is the bacillus of malignant *œdema*; the organism dealt with by me agrees, so far as can be ascertained, with the form to which that title was first applied by Koch, and I seem therefore constrained to call it by that name.

Koch (1881 17) and Gaffky (1881 9), who worked at this organism at the same time, were familiar with Rauschbrand, and distinguished malignant *œdema* from it on morphological grounds and on growth characters in the animal body, also upon a few clinical symptoms in guinea-pigs, such as the much slighter capacity for forming gas. These distinctions as far as they go are quite sound and are still held. Koch and Gaffky's descriptions are lacunar and incomplete, which is not a matter for surprise when the date of their work (1881) is borne in mind.

They give no cultural reactions, as they never succeeded in cultivat-
CERTAIN ANAEROBES ISOLATED FROM WOUNDS. 347

ing the strains. Jensens (1903) had an organism of this same type, and describes its characters in the animal body, and also states that it peptonises milk and that it has a woolly colony upon agar. Thus these early workers are as far as they go in accord with von Hibler's types, and he seems justified in using the name malignant oedema of Koch for his strains.

The organism isolated by myself agrees in no essential point with the _Vibrion septique_ culture, which was very kindly sent me by Dr. Roux (see Table I.). It must, however, be admitted that there seems to be some confusion about the characters of the _Vibrion septique_, as Jungano and Distaso (1910) describe an organism under this name which liquefies serum and digests milk. The _Vibrion septique_ sent to me clots milk without digestion and does not liquefy serum. It seems, in fact, to be closely allied if not identical with the Rauschbrand bacillus, an opinion held by Dr. Nicolle (1915) as the outcome of a number of serological tests.

It may be objected that, owing to the low pathogenicity for guinea-pigs of these proteolytic strains which I have isolated from wounds, they should be classed as _B. putrificus_ (B. cadaveris sporogenes). _B. putrificus_ and the bacillus of malignant oedema (Koch) agree according to the literature in all their cultural reactions and in their growth in colonies. The distinctions are that _B. putrificus_ is described as being markedly an endsporing organism of the long slender drumstick type with round spores, whereas malignant oedema (Koch) is said to have central and subterminal spores, only showing occasional endsporing individuals. The organism obtained by myself shows chiefly central and subterminal spores—the few endsporing individuals seen are attached to comparatively short stout rods and have oval spores and would not be described as "drumstick."

A further distinction is that _B. putrificus_ is described as non-pathogenic, and malignant oedema (Koch) as pathogenic, but it is admitted that the virulence of all these anaerobic types is variable. Thus von Hibler states that virulent malignant oedema (Koch) strains may lose their pathogenicity entirely under cultivation; he also states that _B. cadaveris sporogenes_ (which he realises to be a synonym of _B. putrificus_) may occasionally produce a fatal infection in guinea-pigs. Moreover, he isolates a bacillus which he calls _B. cadaveris sporogenes_ as the only anaerobe present in material taken six hours ante-mortem from a fatal case of gas gangrene occurring in a patient, consequent upon a bad fracture of the leg. It therefore seems that the distinction in regard to pathogenicity is not sufficiently sharp, and the differential diagnosis is apparently being made upon the morphology. For these reasons I have considered that, as the proteolytic forms isolated from these wound cases agree with the _B. malignant oedema_ of Koch in morphology, character of colony, and all cultural reactions, and that without any care having been taken to promote or retain the virulence
they are able to produce gangrenous lesions in guinea-pigs (though the
dose is admittedly large) after twelve to fourteen months' cultivation,
they are to be classed as representatives of the malignant edema
(Koch) type.

It may be pointed out, in regard to variations in virulence in the
anaerobes, that avirulent strains of undoubted tetanus are not
infrequent, and that Rauschbrand even is described as losing its
virulence. Again Welch in his pioneer work with *B. aerogenes
capsulatus* found his strains to be frequently non-pathogenic. In
my own experience, *B. perfringens* is uniformly pathogenic in
six strains isolated from wounds, and non-pathogenic in the seventh.
Therefore it seems that pathogenicity in anaerobes under cultivat-
on cannot be made the critical point of a differential diagnosis.
I may add, in conclusion, that I am not concerned with the
upholding of the specific distinction between *B. putrificus* and
malignant edema (Koch). *B. putrificus* may or may not be a true
species. I am, however, clear that the organism isolated by myself is a
well-defined entity and that it agrees with the malignant edema
strains (Koch) described at length in von Hibler's book. I also do not
defend the suitability of the title chosen by Koch for his bacillus; I
am none-the-less under the necessity of making use of it. The scope of
this paper does not admit of a more complete discussion of the
literature; a list of the authors consulted is, however, appended.

APPENDIX.

**Cooked Meat Medium.**—Eight ounces of bullock's heart, minced very
finely and then ground in a mortar; add 8 ounces of tap-water and heat
slowly, so as to cook the meat thoroughly; add normal sodium hydrate until
the mixture is alkaline to litmus. Divide into tubes and autoclave.

**Alkaline Egg Fluid.**—This is a modification of Besredka's medium. The
yolk of one egg and the whites of two are beaten up in a beaker; add 6 c.c.
of normal sodium hydrate; add 500 c.c. of tap-water by degrees. Heat very
slowly to 95° C., keeping the mixture for this temperature for about an hour or
longer; filter through cotton wool and muslin. Divide into tubes and autoclave at 115° for twenty minutes. This egg fluid may be added to
ordinary nutrient broth in the proportions of about 1 to 5. The egg fluid
must be added to the broth when both the fluids are cool. The egg fluid may
be added to agar at 50° C.

All the sugar media should be made up with nutrient broth instead of
peptone water. The anaerobes do not grow sufficiently well upon peptone
water.

**NOTE.**

Since writing the foregoing, I find that Hibler rx upon being rapidly sub-
cultured and when in a vigorous condition is capable of attacking milk in
forty-eight hours and may produce a more solid clot than that described above.
The fermentation of starch may also become more intense.
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