THE BACTERIAL FLORA OF WAR WOUNDS: INFLUENCE OF TREATMENT.

BY

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THE wounds of war differ from the majority of wounds in civil life in that they contain a very large number of bacteria, resulting in prolonged suppuration, slow healing, and deforming cicatrization. In no industrial wounds, except a limited number occurring in agriculturists, does soil contamination play such an important part as in the wounds of war, and as the bacteria of the soil figure largely in the flora of war wounds, a comparative study of the bacterial flora of the wounds extending over long periods may throw some light on the benefit of one or other method of wound treatment, and suggest a direction in which effort should be concentrated.

From the commencement of the war I have made routine bacteriological examinations of the convoy cases arriving in certain home hospitals; these examinations have been conducted in a uniform manner throughout. The routine adopted has been (a) the examination of film preparations

DIAGRAM I.—Percentage incidence of spores (shaded), bacilli (black), and cocci (white), in Gram-stained films. Periods of six months, 1915-17.

of the discharge material stained by the Gram method, (b) the cultivation of the discharge material on (1) glucose broth, (2) sterilized milk, (3) minced autoclaved meat. The cultures were examined in the ordinary routine manner and where necessary plated out, or the cultures carried far enough to determine the various groups in which the organisms are scheduled. A considerable amount of effort was expended to commence with in perfecting anaërobic apparatus, and the results and methods have been published elsewhere. Certain general conclusions regarding the bacteriological flora of wounds have been reached. In the early part of the war, especially in the winter of 1914-15, many wounds arriving in home hospitals were extremely septic, sloughing, and emitted an offensive putrid odour. At intervals during the succeeding years a slight return to this condition has occurred from time to time, during periods of stress, but the general physiological character of the wounds has undergone a steady and progressive improvement. In the early period the number of spore-bearing organisms found in films made from the wound discharges was large. Three types of sporing organisms were observed: (1) Bacilli with a central spore, staining deeply by Gram's method; (2) bacilli containing a sub-terminal spore; (3) bacilli with a rounded or oval end-spore. In the more putrid wounds all three classes were represented, but mainly the third, whereas in the cases of acute localized oedema with bubbles of gas in the discharge and, in a few cases, acute gas gangrene spreading from the

wound, the main class of organism present was the central spored bacillus. During 1916 and the first half of 1917 these organisms were less often met with; during the last half of 1917 a limited number only were observed.

Diagram I shows the relative percentage incidence of the various classes of organism met with in the pus films. The cases are collected into periods of six months, commencing with 1915, and the number of cases with spore-bearing organisms shows an extraordinary and progressive diminution, except for a slight rise during the second half of 1916. These relative proportional diagrams do not convey the intensity of the infection in any given wound, but merely the percentage of cases in which spore-forming bacteria were found in the pus films. The other two columns represent—the white column cocci, both streptococci and staphylococci, and the black column bacilli, both Gram-positive and Gram-negative. During the first two years Gram-negative bacilli belonging to the *coli* and *proteus* groups were frequently met with, but the incidence of these organisms, especially the *proteus* group, has diminished during the last nine months. The cases from which these examinations are made were all examined within twenty days of wounding. About 33 per cent. were examined within the first week; this proportion has been maintained throughout, so that the groups of cases remain fairly comparable. The results of treatment appear, therefore, to have progressively diminished wound infection, but it by no means follows that the absence of bacteria in the films of the discharge is indicative of the freedom of such wounds from bacterial infection.

Diagram II shows how far this divergence exists; in this diagram the proportional incidence of bacteria in the

DIAGRAM II.—Percentage incidence of anaerobic bacilli in wounds 1 to 20 days old. First examination on admission in home hospitals. Black = Saccharolytic group, type *B. perfringens* (*welchii*) (cultivation). White = Proteolytic group, type *B. sporogenes* (cultivation). Shaded = End-spore group, type *B. tetani* (cultivation).

wounds obtained by cultural methods is given in percentage proportions. The columns relate to three types of anaerobic bacteria: the black, the saccharolytic group, of which *B. perfringens* or *B. welchii* may be taken as the type. The white column, bacilli of the proteolytic group, of which *B. sporogenes* or the bacillus of malignant oedema may be taken as the type; whilst the shaded column indicates the number of organisms forming end spores of the tetanus and other allied groups. It must not be supposed that this class represents tetanus bacilli; it merely indicates the large class of end-sporing bacilli to which the tetanus bacillus belongs.

During the first half of 1916 there was an apparent diminution in the number of sporing anaerobes isolated from the wounds, but from this period forward to the end of 1917 the incidence again rises steeply. From 1915 to June, 1916, the number of proteolytic and saccharolytic organisms remains about the same. A considerable rise occurs in the second half of 1916, and the wounds from this period remain at a higher level of infection, the only diminution being a decrease in the number of proteolytic organisms in the second half of 1917. It is freely admitted that the number of cases examined is extremely small in relation to the total number of wounded. On the other hand, the series has been entirely performed by one observer and according to a constant routine. A possible

explanation of the smaller number of infected wounds culturally up to June, 1916, may perhaps bear some relation to the larger number of bullet wounds, and there is a possibility that from 1916 onwards shell wounds have figured more largely, causing a deeper infection of the tissues.

A comparison of Diagrams I and II points very strongly to the inadequacy of simple film examination in determining the bacterial content of any given wound. It must be pointed out in relation to Diagram II, as to Diagram I, that the percentage occurrence of the groups indicated in the table is no criterion of the mass extent of the infection, as the cultivations were always re-examined at intervals for fourteen days to allow of the growth of any organisms that were present. The diminution in the flora of the wounds, as estimated by film preparations given in Diagram I, together with the apparent increase in the numbers of cultivable bacteria given in Diagram II, suggested some influence which, at any rate, was diminishing the mass infection of the wound. Unfortunately, it is only since June, 1917, that the medical field cards have been available; before this date no information was forthcoming as to the earlier treatment of the wound except by hearsay. From June, 1916, the field cards give a record of the number of wound excisions performed in the casualty clearing stations, as a rule within twelve hours of wounding; it was thought, therefore, that a comparison of the percentage flora in excised wounds and non-excised wounds might give some information as to the possible cause of the diminution.

In Diagram III I have therefore collected the percentage incidence of anaërobic flora from 100 excised wounds examined from June to December, 1917, and of 100 non-excised wounds examined between January and June, 1917, and a further graph of the total number of wounds examined from June, 1915, to June, 1917. There is thus a total percentage of all wounds examined within twenty days of injury up to June, 1917, and the percentage in 100 wounds which have been excised and 100 wounds occurring in the immediately preceding six months, of which no notes are available as to the original operation, if any. The columns in Diagram III are: First, spore-bearing organisms in films from wound discharge; black, the saccharolytic group, determined by cultivation; white, proteolytic group, determined by cultivation; dotted, end-sporing bacilli. It will be seen at once that in the 100 excised wounds all four classes are greatly diminished. Not only have the spore-bearing organisms in direct films diminished, but the spore-bearing bacilli obtained by cultivation also show a considerable diminution, whilst both proteolytic and saccharolytic groups are diminished, the proteolytic to the greatest extent. It may therefore be fairly inferred that the immediate excision of wounds performed at the casualty clearing stations has a very great influence in diminishing the number of bacteria cultivable from the wounds five to twenty days afterwards.

DIAGRAM III.—Percentage incidence of anaërobic flora in excised wounds compared with non-excised wounds. Shaded = Percentage of wounds with sporing bacilli in the pus films (films from pus). Black = Percentage of wounds with saccharolytic group (cultivation). White = Percentage of wounds with proteolytic group (cultivation). Dotted = Percentage of wounds with end-sporing bacilli (cultivation).

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Influence of Antiseptics on the Bacterial Flora of Excised Wounds.

The possible influence of the antiseptic used on the diminution of bacterial flora naturally arises in connexion with these wounds. The 100 excised wounds were therefore examined carefully to determine if any influence

could be traced to the antiseptic used. Fifty of the wounds had been treated with hypochlorite, with or without Carrel tubes; forty had been treated by bipp. The remaining ten had various forms of antiseptics, including flavine and brilliant green. The two chief antiseptics in use were hypochlorite and bipp. On examining the percentage of the flora in each case there was no difference whatever attributable to either antiseptic. In each class—hypochlorite and bipp—the incidence of sporing bacteria and *Bacillus welchii* was 30 per cent.; for the proteolytic type 29 and 31 per cent. respectively. If either of these two antiseptics had any specific action in reducing one or other variety of bacterial infection, one would have expected it to show in a series of wounds treated in the same manner. So far, no sufficient number of cases treated with no antiseptic from the moment of excision is available.

Certain practical disadvantages are associated with both these antiseptics.

1. Hypochlorite: Cases treated with Carrel tubes rarely arrive in home hospitals with the tubes in the position in which they were originally placed, and on the rare occasions on which they are still in the wound the tubes are generally so blocked as to render them entirely useless. On the other hand, as the Carrel tubes are passed through the bandages and fresh hypochlorite is passed down the tube, some of it finding its way into the wound or into the dressing, the chief advantage appears to be that the bandages are not removed in the ambulance train and the case submitted to the chance of fresh infection.

2. Bipp: A certain number of wounds arrive with so much bismuth inserted that it partially obscures the finer structure in fractured bones. A certain number of cases—10 per cent. of 100 cases treated with bipp—have shown symptoms of poisoning due to bismuth—blue line of the gums, blue tongue, and blue patches on the buccal surface of the cheeks, gastritis, diarrhoea and vomiting. Three instances have been seen of iodine poisoning with mental symptoms. In cases of poisoning attributable to the bipp very large quantities had been made use of.

Latent Infection.

This has proved a very common sequela, and has been observed principally in certain classes of wounds.

1. *Flesh wounds*, especially penetrating, lacerated wounds. In these wounds histological examination of the tissue in the immediate neighbourhood of the wound shows changes similar to those associated with gas gangrene, and described in my previous papers,¹ and also the type of muscle degeneration described by McNee in acute gas gangrene. Reference to Diagram III demonstrates that organisms belonging to the saccharolytic gas-forming group are extremely common. There is overwhelming evidence that a large proportion of wounds suffer from incipient gas gangrene, but that the process is arrested by the action of the tissues. I have already published photographs of tissues showing active phagocytosis in incipient gas gangrene.²

2. *Bone injuries*.—Latent infection, as distinct from persistent local sepsis, is more common in bone injuries than in flesh wounds. Comminuted fragments have rarely been found united, even after long intervals, and comprise by far the greater number of sequestra removed at subsequent operations. The sequestra removed from such cases—six, nine, twelve, and eighteen months after the original injury—often show the sharp edge of the original fracture. Radiographical examination and histological examination, together with bacteriological observations, show two main forms of sequestra—(a) hard, (b) soft. The softening sequestra appear to be bacteriologically related to *B. welchii* and the streptococcal groups, the hard sequestra more often to the proteolytic groups, *B. sporogenes*. General local loss of opacity to the x rays is associated with loss of lime salt in the bone, and is apparently associated with the saccharolytic group, though no definite acid reaction occurs in the tissues owing to the action of the buffer substances. Histological examination in latent infection, persistent infection, and closed cavities containing metal fragments or fragments of bone, show scattered organisms in small clusters enclosed in a wall of fibrous tissue. The tissue in the immediate neighbourhood of such bacterial nests is often normal in structure. In ankylosed joints which have been excised after complete healing of the external wounds bacteria have invariably

been found sequestered in the partially destroyed cartilages. Infected areas are met with here in close proximity to normal tissue, and more organisms have been found at the extreme limits of the affected and damaged areas than in the centre of fibrous degenerated tissue. One of the difficulties attendant on subsequent operations on joints, especially excision, is the secondary suppurations which undoubtedly arise from these loculi containing remnants of the original infection.

*Wounds Entirely Healed but Breaking Down after
a Long or Short Interval.*

Many wounds have been examined belonging to this class; they are frequently wounds involving bone, often with very slight bone involvement and no fracture. Histological and bacteriological examination give the same findings, whilst the penetration of the bacteria into the bone extends a considerable distance either side of the original lesion. These wounds break down again after trivial injury or over-exercise. A histological examination of tissue excised near such wounds shows the infection mainly at the junction of the normal and fibrous tissue. The outcome of bacteriological observations on a large series of wounds of latent infection, together with histological examination of excised tissue, points to the physiological action of anaërobic bacteria rather than to general systemic toxæmia as the underlying cause of the persistent infection. The most persistent organism is the *B. sporogenes*, which is in itself of low pathogenic power. It can, however, survive for a long time and in culture tubes, when associated with streptococci or organisms of the *coli* group, and undergoes cyclic development, spores forming and germinating slowly but persistently, with the result that the material becomes ultimately entirely digested. In cases heavily infected with the *B. sporogenes* persistent local oedema has been observed. Histologically, minute general thrombosis of vessels in the immediate vicinity of the wound is common; the thrombus may even spread up through the smaller vessels and project into the larger. Histological examinations of these cases show that the "splash" effect produced in the soft tissues by the velocity of the projectile extends over a considerable distance, that numerous radiating cracks in the tissue occur which become the seat of hæmorrhage. The blood clot formed contracts, associated with the discharge of serum from the wound, coincidentally the bacteria make their way into the cracks, doubtless by capillarity, and subsequently become shut off from the main wound cavity by fibrous tissue and remain latent. There is some indication that this fact has an important bearing on the practical question of the movement of wounded men. It certainly has a very practical bearing on the deep infection of the wounds.

Summary.

In summarizing my observations on wounds in home hospitals from 1915 to the present time it appears—

1. That the mass infection has diminished progressively, but that
2. The persistence of anaërobic bacteria has not undergone similar qualitative reduction.
3. That by the use of the two antiseptics of which statistics have been available (bipp and hypochlorite) no great diminution in anaërobic flora has been effected.
4. That latent infection as opposed to persistent infection is of great importance in "subsequent operations," and that latent infection is determined by the treatment of the case in the first week of the injury, possibly in the first three days.
5. That in latent infection organisms are found in close proximity to healthy tissue and therefore in a region likely to become the site of an incision in a subsequent operation.
6. That bone fragments split off at the original injury invariably become sequestra, probably due to the proteolytic action of the bacteria rather than to destruction of blood supply.
7. That of the various methods which have so far been made use of in the immediate treatment of wounds, immediate excision of the lacerated tissues is apparently the only one that has produced a marked diminution in the anaërobic flora of the wounds.

REFERENCES.

- ¹ *Lancet*, July, September, December, 1916. ² *Ibid.*, September, 1916.

NOTES ON VIBRION SEPTIQUE.

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THE following is a brief account of the anaërobic organism known variously as the *Vibrion septique* and the bacillus of Gohn and Sachs; the name *B. oedematis maligni* has also been used for it.

The conflicting accounts of early writers and the very real difficulty due to the close symbiotic association of widely differing species of anaërobic has obscured our knowledge of this type. *Vibrion septique* is very easily passed over in mixed culture, so that its presence has often escaped the notice of workers dealing with wound infections. I propose to use the name *Vibrion septique*, under which I include the bacillus of Gohn and Sachs; *B. chauwei* is an allied type, but is a different organism.

The name *Vibrion septique* is here applied to a group of strains which are in complete agreement with each other in (a) morphology; (b) cultural characters, including the reactions upon various sugar media; (c) pathogenicity and reaction in the animal body. They do not, however, fall into one group upon the agglutination reaction.

From the trend of modern research it is becoming clear that in many cases the organisms responsible for any given pathological condition must be looked upon as a series of very closely allied types, which can in certain instances be separated from each other by various slight *differentia* and notably by the agglutination reaction. It may eventually be useful to subdivide the group into subgroups, possibly with specific names. It is proposed to deal with the agglutination reactions of *Vibrion septique* in another publication. In general, it may be said that in working with anaërobic it is premature to conclude that a strain represents a separate species because it produces specific agglutinins.

The following is a list of the strains studied:

1. *Vibrion septique*, Pasteur Institute strain.
2. *B. R.*, Pasteur Institute strain.
3. *V. septique*, strain "Amatzi"; derived from blood culture; from Dr. Weinberg.
4. *V. septique*, strain "Cairny"; from fatal case of gas gangrene.
5. *V. septique*, strain "Summers"; blood culture from case of gas gangrene.
6. *V. septique*, strain "Dynes"; from fatal case of gas gangrene.
7. *V. septique*, strain "Prior"; from case of gas gangrene.
8. *V. septique*, strain "Alexander"; from hæmorrhagic blister fluid in a case of gas gangrene.
(Strains 4 to 8 were supplied by Captain Stokes.)
9. *V. septique*, strain "J. I."; from case of gas gangrene isolated by Miss Hempl.
10. *V. septique*, strain "James"; from case of gas gangrene.
11. *V. septique*, strain "Brissons"; from case of gas gangrene.
12. *V. septique*, strain 141; from case of gas gangrene.
13. *V. septique*, strain 133; from case of gas gangrene.
(Strains 10 to 13 were supplied by Captain Henry.)
14. *V. septique*, strain 227; from wound of patient forty-nine days after wounding; no symptoms of gas gangrene.
15. *V. septique*, strain 145; from wound of patient seven days after wounding; no symptoms of gas gangrene.
16. *V. septique*, strain "Jerral"; from wound of patient without symptoms.
17. *V. septique*, strain "Bedford"; amoebic abscess of brain; material from Captain Armitage.
18. *V. septique*, "Milk" strain, isolated from milk.
19. *V. septique*, "Milk Con." strain, isolated from milk.
20. *V. septique*, strain "George A."; from case of gas gangrene developing upon operative treatment of a healed wound.
21. N. I., *V. septique*; strain isolated by Miss Hempl from wound of patient without symptoms of gas gangrene.

The following were studied for comparison:

22. *B. chauwei*; from case of black quarter; from Sir John McFadyean.
23. *B. chauwei*, culture isolated by Dr. MacIntosh from Rauschbrand muscle from Denmark.
24. *B. oedematis*, strain "Jolly"; from Dr. Weinberg, from case of Dr. Dalyell.
25. *B. oedematis*, strain "Delombre"; from Dr. Weinberg.

I have much pleasure in expressing my cordial thanks to the many colleagues to whom I am indebted for material from cases in their charge, or who have most generously placed their cultures at my disposal. My thanks are due to Dr. Roux, Dr. Weinberg, Dr. MacIntosh, Miss Hempl, Captain Armitage, N.Z.M.C., and Sir John McFadyean. I am quite especially indebted to Captain A. Stokes, D.S.O., R.A.M.C., and to Captain Henry, R.A.M.C., for much valuable material and for kind co-operation during the course of the work.

Morphology.

The *Vibrion septique*, like many anaërobic, has a rather wide range of variation in morphology according to the circumstances in which it develops.

Typically, in a young culture in meat medium it appears as a Gram-positive rod of varying length considerably thinner than