STUDIES IN WOUND INFECTIONS:
ON THE QUESTION OF BACTERIAL SYMBIOSIS
IN WOUND INFECTIONS.

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FOR many years it has been recognised that one species of micro-organism may exercise a helpful action on the proliferation of another when the two are grown together in the same culture medium. Earlier examples of this type of symbiosis—the term usually applied to this phenomenon—are: (1) the growth of an anaerobic bacillus in association with an aerobe, (2) the growth of such a bacillus as *B. influenza* in association with either staphylococcus or *B. xerosis*.

With regard to the first example given above in which an aerobic micro-organism enables an anaerobic one to grow freely, the explanation usually given is that the aerobe in its growth absorbs the oxygen, thus making the conditions favourable for the proliferation of the anaerobe. This principle has been frequently employed in the cultivation of anaerobes and numerous ingenious methods have been devised for thus utilising the oxygen-absorbing properties of *B. subtilis* and other bacteria.

In respect to the second example given in which the growth of *B. influenza* is assisted by the presence of another micro-organism such as staphylococcus no such simple explanation can be given, but it is certain that the staphylococcus furnishes a substance which enables the influenza bacillus to flourish on a medium on which it will not grow alone, and also enables it to maintain its vitality for long periods.

In connexion with septic wounds of the present war the question of symbiosis may assume very considerable importance. In all these wounds the bacterial flora is a varied one consisting as it does of anaerobic and aerobic, spore-bearing and non-sporing bacteria. As has been shown by Fleming,¹ the primary infection of the wounds is a faecal one. At first there is a phase in which the anaerobic spore-bearing bacilli (*B. perfringens* and organisms of putrefaction) flourish together with streptococci, *B. proteus*, and diphtheroids. The anaerobic bacilli as time goes on gradually disappear, and ultimately there remain streptococci, staphylococci, diphtheroid organisms, and, at times, *B. proteus*.

The microbes (apart from *B. tetani*) which directly cause the most serious infections are, in the early stages of the wound, *B. perfringens*, possibly *B. malignant edema* and streptococci, while in the later stages streptococci are responsible for almost all the serious complications. Although the other microbes do not usually cause serious complications, their presence in the wound becomes an important matter if it can be shown that they may act as symbiotic stimulants to the growth of the more dangerous members of the bacterial flora. The experiments detailed below indicate that this is the case.

In order to approximate the conditions of the experiment to those obtaining in wounds the culture medium usually employed was human serum, either unaltered or serum to which sufficient trypsin had been added to neutralise the antitryptic power. The *raison d'être* of this procedure was that Wright has shown that, according to their situation, the bacteria proliferating in a wound have two varieties of culture media from which they obtain their nutriment.

(i.) Those proliferating in the cavity of the wound are supplied with a fluid medium which consists of the blood fluids altered to a varying degree by the breaking down of the extravasated leucocytes—a process which leads to the liberation of a large amount of trypsin. According to the amount of trypsin thus set free the antitryptic power of these blood fluids is either diminished, completely neutralised,

or the resulting fluid is even—and this is by no means an unfrequent stage—rendered strongly tryptic. All these changes render the blood fluids a much more suitable pabulum for bacteria, for it has been shown that neutralisation of the antitryptic power of the blood serum completely destroys all the antibacterial properties of the blood, and the presence of free trypsin together with albumens obviously leads to the formation of digestion products which are the best of all foods on which to cultivate bacteria.
(ii.) Those bacteria proliferating in the lymph spaces of damaged but still living tissues are bathed in a fluid consisting of lymph altered to a much slighter extent, according to the time it has been exuded from the vessels, the number of leucocytes it contains, and other, at present, unrecognised factors.

PART I.—Do the Other Microbes Present in a Wound Aid the Growth of *B. perfringens*?

Fleming² in some preliminary experiments has shown that staphylococci, streptococci, and *B. proteus* when grown in association with *B. perfringens* caused an increased growth of this latter micro-organism.

Before proceeding to give the details of the various experiments the following points concerning the growth of *B. perfringens* in culture should be mentioned.

B. perfringens when planted into fresh serum and incubated under anaerobic conditions will only grow when the sowing is a heavy one.

Wright has shown that if the antitryptic power of the serum is neutralised by the addition of trypsin or its alkalinity is neutralised with an acid growth occurs with a much lighter sowing.

When growth takes place in serum a very large quantity of gas is formed, so that four to five volumes of gas may be produced from one volume of serum. This circumstance affords a convenient index of the presence and also of the amount of growth.

Experiment I. Influence of staphylococci and streptococci on growth of B. perfringens in milk.—A broth culture of *B. perfringens* was diluted with broth 10-fold, 100-fold, and by 10-fold steps to a million-fold dilution. 40 c.mm. of each dilution was planted into (a) milk, 1 c.c.; (b) milk, 1 c.c. + 20 c.mm. staphylococcus broth culture; (c) milk, 1 c.c. + 20 c.mm. streptococcus broth culture. These tubes having been incubated for 24 hours were examined for growth, which was indicated by the formation of the typical clot and gas formation. The results are shown in Table I.:

No. of tube and micro-organisms planted.		Dilution of <i>B. perfringens</i> broth cultures.					
		1/5	1/50	1/500	1/5000	1/50000	1/500000
(a)	<i>B. perfringens</i> only	Gr.	Gr.	0	0	0	0
(b)	" " + staphylococcus	"	"	Gr.	Gr.	Gr.	Gr.
(c)	" " + streptococcus	"	"	"	"	"	"

Gr. = growth.

Experiment II. Influence of diphtheroid bacilli on growth of B. perfringens in serum (with and without the addition of trypsin).—In this experiment pieces of 6 mm. bore glass-tubing about 15 cm. long and sealed at one end were used as culture tubes. Into these miniature test-tubes the culture materials and micro-organisms were introduced and then melted vaseline was run on to the surface to form a column about 2.5 cm. high. The index of growth was gas formation evidenced by the vaseline being pushed up the tube. Dilutions of *B. perfringens* broth culture having been made, as in Experiment I., 10 c.mm. of each was planted into 300 c.mm. of serum. Another 10 c.mm. of each dilution was planted into a similar tube of serum which had been thickly

No. of tubes and micro-organisms planted.		Dilution of <i>B. perfringens</i> culture.							Culture medium.
		1	1/5	1/50	1/500	1/5000	1/50000	1/500000	
(a)	<i>B. perfringens</i> alone.	Gr.	Gr.	0	0	0	0	0	TS
(b)	" + diphtheroid.	"	"	Gr.	Gr.	Gr.	Gr.	Gr.	
(c)	<i>B. perfringens</i> alone.	0	0	0	0	0	0	0	US
(d)	" + diphtheroid.	Gr	Gr.	Gr.	Gr.	Gr.	Gr.	Gr.	

Gr. = growth. TS = trypsinised serum. US = unaltered serum.
sown with a diphtheroid bacillus. The experiment was then repeated, using serum to which sufficient trypsin had been added to neutralise the antitryptic power. The results are shown in Table II.

1 THE LANCET, 1915, ii., 638.

2 THE LANCET, 1915, ii., 376.

Two and a half hours after planting these cultures showed gas formation in the first tubes of series (b) and (d) and in four hours the amount of gas produced in the first tube of series (b) was equal in bulk to the culture fluid. Such rapid gas formation has not been noted under any other condition.

Experiment III. Do all the diphtheroids commonly present in wounds stimulate the growth of B. perfringens to the same extent?—Fresh human serum was divided into portions and each portion was planted thickly with a strain of a diphtheroid bacillus. The diphtheroid bacilli employed were representatives of the various groups frequently found in wounds.

Two hundred and fifty c.mm. of the serum containing the diphtheroid bacillus were distributed into miniature test-tubes, as described in Experiment II., and to each were added 50 c.mm. of dilutions, as shown below, of a *B. perfringens* broth culture (four days old). Vaseline was then poured on the top of the fluid and the tubes were incubated for five days. The results are given in Table III.:

TABLE III.

Strain of diphtheroid used.	Dilution of <i>B. perfringens</i> culture.			
	1:1000	1:10000	1:100000	1:1000000
Forrest V.	Growth	Growth	0	0
Cation I.	"	"	0	0
Ford I.	"	"	0	0
Ryan I.	"	"	Growth	0
Beavan I.	"	"	0	0
Moore III.	"	"	0	0
Beavan IV.	"	"	Growth	0
Pretty V.	"	"	"	0
Driscoll II.	"	"	0	0
Control*	0	0	0	0

* Without diphtheroid, *B. perfringens* only.

From the above table it will be seen that these diphtheroids all stimulate the growth of *B. perfringens* in serum and approximately to the same extent.

Experiment IV. Influence of a coliform bacillus and B. proteus on growth of B. perfringens in serum.—Similar small tubes to those used in former experiments were filled in as follows:—

- Tube A. Serum 1 c.c. + *B. perfringens* emulsion 10 c.mm.
 " B. Serum 1 c.c. + *B. perfringens* emulsion 10 c.mm. + coliform bacillus emulsion 10 c.mm.
 " C. Serum 1 c.c. + *B. perfringens* emulsion 10 c.mm. + *B. proteus* emulsion 10 c.mm.

These tubes were incubated anaerobically for 18 hours and the number of *B. perfringens* estimated by counting against the number of the red blood corpuscles (Wright's method). The results obtained were as follows:—

B. perfringens per c.c.: Tube A, 62 millions; Tube B, 146 millions; Tube C, 357 millions.

Experiment V. To ascertain, when two microbes are grown in symbiosis, whether both are stimulated to increased proliferation.—The micro-organisms employed in this experiment were *B. perfringens*, staphylococcus, and streptococcus. Miniature test-tubes containing 1 c.c. of the fluid from an ovarian cyst were planted with cultures as shown below. After incubation under anaerobic conditions for two days, the number of microbes was estimated by counting them against the number of red blood corpuscles in a film (Wright's method) and also, in the case of the aerobic micro-organisms, by diluting and planting a known quantity of a dilution on to plates, which were then incubated under aerobic conditions, the resulting colonies being counted after a suitable interval. The results are given in Table IV:—

TABLE IV.

No. of tube.	Micro-organisms planted into the ovarian cyst fluid.*	No. of microbes per c.c. estimated by Wright's method after incubation.	No. of cocci obtained by plating 20 c.mm. of the fluid diluted 500 fold, after incubation.
I.	<i>B. perfringens</i> emulsion.	360 million.	—
II.	<i>B. perfringens</i> emulsion + staphylococcus emulsion.	<i>B. perfringens</i> 433 million, staphylococcus 880 million.	Innumerable colonies of staphylococcus.
III.	Staphylococcus emulsion.	400 million.	About 1500 colonies.†
IV.	<i>B. perfringens</i> emulsion + streptococcus emulsion.	<i>B. perfringens</i> 620 million, streptococcus 140 million.	265 colonies of streptococcus.
V.	Streptococcus emulsion.	50 million.	4 colonies of streptococcus.

* In each instance 10 c.mm. of emulsion was planted.

† Certainly less than half the number of colonies obtained from Tube II. This experiment shows conclusively that when *B. perfringens* is grown in symbiosis with either staphylococcus or streptococcus the stimulation to increased growth is mutual to both species of bacteria.

Experiment VI. Influence of streptococci and staphylococci on the growth of B. perfringens in serum.—In this experiment the technique was modified by growing the cocci in serum for three days before planting the *B. perfringens*. Three small test-tubes, each containing 1 c.c. serum, were taken, one was planted with streptococcus, another with staphylococcus, while the third was left uninoculated. All three tubes having been incubated together anaerobically for three days an equal amount of *B. perfringens* culture was added to each. After a further incubation for 18 hours the number of *B. perfringens* were estimated by Wright's method.

- Tube I. (staphylococcus + *B. perfringens*) contained 120 million *B. perfringens* per c.c.
 " II. (streptococcus + *B. perfringens*) contained 480 million *B. perfringens* per c.c.
 " III. (*B. perfringens* only) contained 62 million *B. perfringens* per c.c.

The above experiments show that the growth of *B. perfringens* is greatly increased when it is grown symbiotically with either streptococcus, staphylococcus, or various diphtheroid bacilli. It might be argued, however, that this increased growth was simply due to some simple action such as the neutralisation of the alkalinity or the antitryptic power of the serum, both of which, as mentioned above, have been shown by Wright to aid the growth of this bacillus. The following experiment shows, however, that the beneficial action of symbiosis with streptococci, diphtheroid, and a coliform bacillus is just as marked in the case when the serum, employed as culture medium, has previously been neutralised either in respect to its alkalinity or its antitryptic power.

Experiment VII.—The technique employed was similar to that used in Experiments II. and III.

TABLE V.

Series A.—In this series the culture medium consisted of serum 7 parts and distilled water 2 parts.

Micro-organisms planted.	Dilutions of <i>B. perfringens</i> culture.						
	1:1	1:10	1:100	1:1000	1:10000	1:100000	1:1000000
1. Control. <i>B. perfringens</i> only	Gr.	0	0	0	0	0	0
2. Diphtheroid bacillus + <i>B. perfringens</i>	"	Gr.	Gr.	Gr.	Gr.	Gr.	0
3. Streptococci + <i>B. perfringens</i>	"	"	"	"	0	0	0
4. Coliform bacillus + <i>B. perfringens</i>	"	"	"	"	Gr.	Gr.	Gr.
5. <i>B. pyocyaneus</i> + <i>B. perfringens</i>	"	"	"	"	"	0	0

Series B.—In this series the culture medium consisted of serum 7 parts and HCl N/10 2 parts (this amount of acid just neutralised the alkalinity of the serum).

1. Control. <i>B. perfringens</i> only	Gr.	0	0	0	0	0	0
2. Diphtheroid + <i>B. perfringens</i>	"	Gr.	Gr.	Gr.	0	0	0
3. Streptococci + <i>B. perfringens</i>	"	"	"	"	Gr.	Gr.	Gr.
4. Coliform + <i>B. perfringens</i>	"	"	"	"	"	"	"

Series C.—In this series the culture medium consisted of serum 7 parts, trypsin solution diluted 6 times, 2 parts.

1. Control. <i>B. perfringens</i> only	Gr.	Gr.	0	0	0	0	0
2. Diphtheroid + <i>B. perfringens</i>	"	"	Gr.	Gr.	Gr.	Gr.	0
3. Streptococci + <i>B. perfringens</i>	"	"	"	"	"	"	Gr.
4. Coliform + <i>B. perfringens</i>	"	"	"	"	"	"	"
5. <i>B. pyocyaneus</i> + <i>B. perfringens</i>	"	"	"	"	"	"	0

Gr. = "Growth" and refers only to growth of *B. perfringens* as evidenced by gas formation in serum.

PART II.—*Do the Various Micro-organisms Commonly Found in Wounds Favour the Growth of Anaerobic Bacilli other than B. perfringens?*

Experiment VIII. Influence of streptococci on the growth of the bacillus of malignant oedema in serum.—A broth culture of malignant oedema was diluted by ten-fold steps up to a million-fold dilution. Of each of these dilutions 40 c.mm. were planted into a series of tubes containing 1 c.c. of serum and also into a series containing 1 c.c. of serum previously planted with streptococci. Vaseline was poured into the

tubes to make a column 2.5 cm. high and the tubes were then incubated at 37° C. for five days. The results then were as shown in Table VI. :—

TABLE VI.

Series of tubes.	Dilution of broth culture of malignant cedema.						
	$\frac{1}{1}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{10000}$	$\frac{1}{100000}$	$\frac{1}{1000000}$
(A) Without streptococcus.	0	0	0	0	0	0	0
(B) With „	Gr.	Gr.	Gr.	Gr.	Gr.	Gr.	Gr.

Gr. = growth.

Experiment IX. Influence of diphtheroid bacilli, streptococci, and staphylococci on the growth in serum of the bacillus of malignant cedema and B. Hibler IX.—A quantity of serum was divided into two portions, one of which was implanted with the bacillus of malignant cedema, the other with B. Hibler IX. These implanted sera were now distributed in two series of minute test-tubes, 0.2 c.c. being added to each. One tube of each series was kept as a control, the others being further implanted with a different micro-organism. Melted vaseline was now poured on to the surface of the fluid and the tubes were incubated at 37° C. After varying intervals the tubes were examined for growth of the anaerobic bacillus which was demonstrated by the formation of gas. The results are shown in Table VII. :—

TABLE VII.

Other micro-organism planted.	Serum planted with the bacillus of malignant cedema.			Serum planted with <i>B. Hibler IX.</i>		
	Time of incubation in days—					
	3	5	9	3	5	9
None	0	0	0	0	Gr.	Gr.
Diphtheroid No. I. ...	Gr.	Gr.	Gr.	Gr.	„	„
„ No. II. ...	„	„	„	„	„	„
<i>Streptococcus faecalis</i> ...	„	„	„	„	„	„
<i>Staphylococcus</i>	„	„	„	0	„	„

From these two experiments it is seen that the ordinary non-sporing microbes found in wounds greatly stimulate the growth of the malignant cedema bacillus and B. Hibler IX., both of which have been said to cause gas gangrene.

PART III.—Do the Other Microbes Commonly Present in a Wound Favour the Growth of Streptococcus?

As streptococcus is the cause of many of the complications, such as cellulitis or septicaemia, which occur even in the earlier stages of wounds and, in the later stages, is the one markedly pathogenic microbe, with the exception of staphylococcus, that is almost constantly present, any condition which favours its growth must be at once recognised as of great importance. In the earlier stages the associated microbes are usually B. perfringens, putrefactive anaerobic bacteria, B. proteus, and diphtheroid bacilli, while in the late stages it is associated with diphtheroid bacilli, B. proteus, and staphylococci.

Throughout these experiments in determining the amount of streptococcal growth the total number of cocci were counted by Wright's method or the number of living cocci were estimated by diluting the culture and plating out a known quantity of a dilution on an agar plate.

Experiment X. Influence of B. perfringens on the growth of streptococcus.

Tube I. 1 c.c. fresh human serum planted with 10 c.mm. streptococcus emulsion.
„ II. 1 c.c. fresh human serum planted with 10 c.mm. streptococcus emulsion + 40 c.mm. B. perfringens broth culture.

After incubation for 18 hours under anaerobic conditions the number of streptococci was counted by Wright's method.

Tube I. (streptococcus only) contained 100 million streptococci per c.c.
„ II. („ + B. perfringens) „ 360 „ „ „

Experiment XI. Influence of B. perfringens on growth of staphylococci and streptococci.—Four tubes, each containing 1 c.c. of serum, were taken. Two of them were planted with B. perfringens, the other two remaining unplanted. After all the tubes had been incubated together for 24 hours under anaerobic conditions an equal quantity of staphylococci culture was planted into one of the unplanted tubes

and into one of the B. perfringens cultures. The remaining tubes were similarly planted with streptococcus. After having been incubated under anaerobic conditions for 24 hours each culture was diluted 1000-fold with broth, and 10 c.mm. of this dilution was distributed on to the surface of an agar plate. These plates were incubated under aerobic conditions, and the resulting colonies of streptococcus or staphylococcus were counted. The results are shown in Table VIII. :—

TABLE VIII.

Tube I. (B. perfringens + staphylococcus) gave about 600 colonies of staphylococcus.
„ II. (Staphylococcus only) gave 64 colonies of staphylococcus.
„ III. (B. perfringens + streptococci) gave about 1000 colonies of streptococci.
„ IV. (Streptococci only) gave 1 colony of streptococci.

When it is considered that both streptococci and staphylococci grow much more readily in unaltered serum than B. perfringens this enormous stimulation of growth is all the more surprising.

Experiment XII. Effect of diphtheroid bacillus on growth of streptococci.—Into each of three small test-tubes 1 c.c. of serum was placed. To the first was added 10 c.mm. of 1000-fold diluted broth culture of a streptococcus; to the second 10 c.mm. of 1000-fold diluted broth culture of a diphtheroid bacillus; and to the third 10 c.mm. of the 1000-fold diluted streptococcus culture and 10 c.mm. of the 1000-fold diluted diphtheroid bacillus culture. The number of living micro-organisms in each tube was then estimated by plating out a known quantity on agar and counting the resulting colonies. The three tubes were then incubated for 10 hours at 37° C., after which the number of living micro-organisms was again estimated by the same method. The results are given in Table IX. :—

TABLE IX.

No. of tube and implantation.*	Content of living streptococci per c.c.		Content of living diphtheroid bacilli per c.c.	
	At time of planting.	After 10 hours incubation.	At time of planting.	After 10 hours incubation.
1. Streptococcus culture ...	13,000	2,800,000	—	—
2. Diphtheroid culture	—	—	2000	5,300,000
3. Streptococcus culture and diphtheroid culture ... }	13,000	120,000,000	2000	2,600,000

* In each instance 10 c.mm. of broth culture diluted 1000-fold.

This experiment shows a very great increase in the numbers of streptococci in Tube 3 where streptococci are grown in association with diphtheroid bacilli. In this tube there was after 10 hours more than 40 times as many streptococci as in an exactly comparable tube where the streptococcus was grown by itself.

Association with streptococci did not appear to favour the growth of the diphtheroid bacilli; on the other hand, it seemed to retard it, as there were only half the number of living diphtheroid bacilli in the tube containing the mixed culture as there were in the tube in which the diphtheroid bacillus only had been grown.

With regard to the mechanism by which the aerobic organisms help the anaerobes the following experiment is of interest.

Experiment XIII.—An ordinary broth culture tube was incubated with a diphtheroid bacillus such as is commonly found in wounds. The cotton-wool plug was cut off short and pushed a little way down the tube. Melted vaseline was now poured on the plug so as to soak into it and to leave a layer of vaseline about 0.5 cm. thick on the top. The tube was put in the incubator, and when it had reached incubator temperature a mark was made at the upper level of the vaseline. As oxygen was absorbed from the tube so the vaseline was sucked down, and at intervals marks were made corresponding with the level of the vaseline.

The height of the column of air in the tube was 87 mm.

In 6 days the vaseline was sucked down 7.5 mm. = 0.09 of total volume
In 10 „ „ „ 13.5 mm. = 0.15 „ „
In 4 months „ „ „ 17.5 mm. = 0.2 „ „

We see, then, that ultimately the amount of gas absorbed was 0.2 of the total volume, which corresponds with the amount of oxygen in air, and the absence of oxygen was confirmed by the introduction of pyrogallie acid and caustic soda, when no further absorption took place and no brown colour was produced.

Further experiments showed that staphylococcus, streptococcus, *B. pyocyaneus*, and some coliform bacteria absorb oxygen to a greater or less extent during their growth.

While this oxygen absorptive power may play some part in the symbiotic action of aerobic bacteria on the anaerobes, yet it cannot be the only explanation, as the experiments cited above show that the stimulant action of the "aerobic" bacteria is exercised when the cultures are grown under absolutely anaerobic conditions.

Summary and Conclusions.

Part I.—The experiments described show that:—

(i.) The proliferation of *B. perfringens* is much more rapid when it is grown in symbiosis with streptococcus, staphylococcus, various diphtheroid bacilli, *B. proteus*, *B. pyocyaneus*, and a coliform bacillus.

(ii.) Such increased growth occurs equally well when the culture medium consists of fresh serum or of serum which has been neutralised either in respect of its alkalinity or its antitryptic power, indicating that the increased proliferation in fresh serum cultures is not due to either of these changes taking place.

(iii.) All strains of diphtheroid bacilli, commonly isolated from wounds, were found to be practically equally efficient in stimulating the growth of *B. perfringens*.

(iv.) Not only were a much larger number of *B. perfringens* found in the cultures grown in symbiosis with these various bacilli after a certain period of incubation, but it was also found that an almost infinitely smaller number of *B. perfringens* would proliferate under these conditions than when pure cultures were made.

(v.) When *B. perfringens* and streptococci are grown in symbiosis a much more abundant growth of both microbes results.

Part II.—The experiments detailed show that:—

(vi.) Nos. (i.), (ii.), and (iv.) of the above conclusions applied equally when the bacillus of malignant œdema or *B. Hibler IX.* was substituted for *B. perfringens*.

Part III.—The experiments described show that:—

(vii.) *B. perfringens* when grown in symbiosis not only with streptococci, but also with staphylococci, leads to an enormous increase in the number of cocci found in the culture.

(viii.) Streptococci grown in symbiosis with diphtheroid bacilli gave a much more abundant growth than when grown in pure culture. The growth of the diphtheroid bacilli in the mixed culture was, however, 50 per cent. less than in the pure culture.

(ix.) The final experiment shows that diphtheroids and many other bacilli have the power of absorbing oxygen to a greater or less extent during their growth, but that this property will not account entirely for the increased proliferation that occurs, when aerobes and anaerobes are grown in symbiosis, since similar mixed cultures placed under anaerobic conditions show quite as marked an increase of growth.

These experiments indicate that bacterial symbiosis may play a very important rôle in wound infections. For as regards gas gangrene, which is caused by the invasion of the tissues by certain anaerobic bacilli, the commonest being the *B. perfringens*, it is well known that very large doses of pure cultures of this bacillus have to be inoculated into animals in order to produce this disease with certainty; again, Wright has shown that to obtain a growth of this bacillus in serum a very heavy implantation is necessary, but if the serum is altered by neutralisation of its alkalinity or its antitryptic power a very much smaller sowing will produce an abundant culture. From these experiments he concluded that the conditions leading to such an alteration of the blood fluids play an important part in the production of gas gangrene.

Taking these facts into consideration, together with many of the clinical aspects of gas gangrene, it would seem apt to compare the action of *B. perfringens* in the production of this disease with a high explosive which is comparatively harmless until the explosive action is started by a detonator. Keeping this simile in view, it is obviously of the utmost importance to ascertain those conditions which act as "detonators" in the growth of this bacillus, and the experiments detailed above show that over and above those factors which have been described by Wright there is another—namely, symbiosis—and that this is in *in vitro* experiments the most important as yet described, since the growth of

B. perfringens obtained in serum under such conditions is not only much more rapid in development and more copious in quantity, but also follows the lightest implantations.

These results would, for example, explain the occurrence of gas-gangrene in those cases where the number of bacilli introduced into the wound at the time of its infliction was comparatively small. They would also explain the frequent occurrence of gas-gangrene in those cases in which after a thorough cleansing and opening up of the wound—a procedure which should reduce the infection to a minimum—free drainage is prevented either by suture or unsuitable dressings. Again, these experiments would explain the almost "explosive" onset of gangrene in some of the severe, heavily infected wounds, for the results obtained in Experiment II., in which, within four hours, gas equivalent to the volume of the serum used as a culture medium was produced, indicate a rate of growth which has never been approached under any other conditions.

These experiments show also that the streptococci multiply much more rapidly when grown in symbiosis with various bacteria, amongst these being the group of diphtheroid bacilli which are present in practically every infected wound from the earliest stage until cicatrization is complete. Such being the case, it is very probable that symbiosis plays an important part in the production of cellulitis and similar complications due to streptococci occurring in the earlier stages of the wound, and also accounts for the common occurrence of the severe inflammatory reactions which so frequently follow operative interference in the later stages of the wound.

CLINICAL TYPES OF SUBTERTIAN MALARIA

AS SEEN IN SALONIKA IN SEPTEMBER, OCTOBER, AND NOVEMBER, 1916.

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DURING last September, October, and November we had an opportunity of seeing over 3600 cases of malaria. The diagnosis of malaria has been founded either on a positive blood finding or on the history of the case, temperature chart, and a definitely hard palpable spleen. In a small proportion of the cases other infections were present in addition. In isolated cases a double infection with subtertian and quartan parasites was found. Owing to the fact that in some 40 per cent. of the cases we were unable to demonstrate the parasites in the peripheral blood stream we are not in a position to give exact figures of the relative amounts of benign and subtertian infections, but there can be no doubt that during this period the percentage of subtertian infections was very high. The relatively high proportion of the negative blood finding is due to the fact that practically all the patients were under the influence of quinine on admission to the hospital and many of them had been several days under hospital treatment before admission to a base hospital. Further, owing to pressure on beds, it was generally impossible, in the less severe cases, to make more than one blood examination.

The majority of the cases were of the simple subtertian, double subtertian, and irregular types of subtertian pyrexia without local manifestations. These cases uniformly responded well to treatment with quinine. The most important group of cases with local symptoms, on account both of relative frequency and of difficulty of differential diagnosis, were characterised by more or less urgent gastrointestinal symptoms. This group could be separated into several more or less definite types.

Dysenteric.—A large number of the cases were sent into hospital with a diagnosis of dysentery. A prominent symptom was the presence of diarrhoea. The great majority of the patients stated they were passing "blood and slime," but this is a mere formula with most of them and of no value in diagnosis. In most of these cases the stools were fluid and feculent, without definite blood or mucus, and they varied