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REPORT OF BACTERIOLOGICAL INVESTIGATION OF TETANUS CARRIED OUT ON BEHALF OF THE WAR OFFICE COMMITTEE FOR THE STUDY OF TETANUS.

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(With 17 Diagrams.)

(*From the Laboratories of the R.A.M. College and the Lister Institute of Preventive Medicine.*)

CONTENTS.

To Major-General Sir DAVID BRUCE, K.C.B., F.R.S.,
Chairman of the War Office Committee for the Study of Tetanus.

SIR,

I have the honour to present the following Report dealing with the bacteriological research which has been prosecuted on behalf of the Tetanus Committee, during the period October 1917–October 1918.

Most of the work herein reported has been carried out jointly by Miss D. M. Cayley and myself.

Miss Cayley is solely responsible for Section VI (pp. 172–195) of the Report, in which is discussed the influence which various dressings and surgical procedures exert upon the anaërobic flora of wounds. The work was arduous and particularly trying, in that the information gained was by no means commensurate with the effort entailed in obtaining it. Miss Cayley is therefore to be congratulated upon her assiduous attention to this task.

The Report is divided into the following Sections:

I. An inquiry into the occurrence of the various types of *B. tetani* in the wounds of men suffering from tetanus.

II. An inquiry into the occurrence of the various types of *B. tetani* in wounds of men showing no evidence of tetanus.

III. A discussion of the facts set forth in Sections I and II.

IV. Experiments carried out *in vitro* to determine whether immune sera, prepared by inoculation of *whole culture* into animals, contained antibodies, other than agglutinins, specific to the "Types."

V. Experiments conducted *in vivo* to examine the problem of *infection* with *B. tetani*, as contrasted with *intoxication* due to absorption of the products of that organism.

The points dealt with in Section V are:

(*a*) The importance of a suitable *nidus* for the development of infection.

(*b*) Relation which the degree and nature of the tissue destruction or debilitation necessary for the inception of infection bears to the development of tetanus, and to prophylaxis by antitoxin.

(*c*) The part played by concomitant infection with micro-organisms other than *B. tetani* in activating or depressing the infective process and the intoxication in tetanus.

(*d*) The immunity conferred by the use of *mono-typical antitoxic* and *anti-bacterial* sera.

VI. The influence which various surgical procedures exert upon infection of wounds due to anaërobic bacteria.

VII. Attempts (*a*) to diagnose tetanus, and (*b*) to determine the "Type" of the infection in cases of the disease by means of an agglutination reaction, using the blood of patients suffering from tetanus for agglutinating stock emulsions of *B. tetani*.

VIII. An inquiry into the question of whether the serological Types are evolved as a result of the residence of the bacillus in the tissues of an individual belonging to one or other of the haemagglutinating groups of men.

IX. A discussion of two cases of abdominal tetanus.

X. The results of examination of faeces for the presence of *B. tetani* in the intestinal contents of

(*a*) Civilians,

(*b*) Men returned from active service.

Throughout each phase of the investigation one object only has been kept in view—the possible application of laboratory findings to the improvement of prophylaxis and therapeutics of tetanus.

Until *mono-typical antitoxic* and *anti-bacterial* sera are available in much larger quantities than is possible when ordinary laboratory animals are used for serum preparation, many of the questions raised by the work described in this Report must remain unsettled.

The investigations discussed in this Report are therefore admittedly incomplete, but the work has, I think, reached a phase in which it may, with advantage, be submitted for discussion.

I have the honour to be, Sir,

Your obedient Servant,

(Signed) WILLIAM J. TULLOCH, M.D., Bt. Major, R.A.M.C.
Lecturer in Bacteriology, The University of St Andrews.

INTRODUCTION.

IN a paper published in the *Journal of the R.A.M.C.*, December 1917, it was demonstrated, that *B. tetani* was susceptible of classification by serological methods into at least three "Types." Since the publication of that paper, a fourth Type has been encountered; but, so far, it has been demonstrated only in five instances—four being from cases of declared tetanus which were not, however, fatal.

The serological classification of *B. tetani* at once suggested the question: "What relationship, if any, do the various Types of the bacillus bear to the causation and pathology of the disease?"

The necessity for an inquiry was insistent, as both the prophylaxis and therapeutics of tetanus might have to be modified in view of the findings obtained.

The investigation was prosecuted as follows:

(*a*) As many cases of tetanus as possible were examined by bacteriological methods, in order to determine the serological Type of the bacillus responsible for the causation of the disease in each instance.

(*b*) In order to control these findings, the frequency with which the various serological Types of *B. tetani* could be obtained from wounds of men not suffering from tetanus, was made the subject of inquiry.

(*c*) Laboratory experiments, both *in vitro* and *in vivo*, were carried out, in order to determine whether crossed immunity to intoxication or to infection with the various Types, or their products, indicated that the typing of the bacilli was or was not of importance in relation to the pathogenesis of the disease and its serum prophylaxis. The question of the degree of passive immunity to *infection* conferred by the injection of antitoxin, naturally called for examination in this connection.

(*d*) The influence which various surgical procedures might have in preventing or lessening mass infection with anaërobes was also investigated. This Section of the work is of special interest and importance, as the most important element in the prevention of anaërobe infections is the surgical procedure employed in the treatment of wounds.

(*e*) The question was investigated as to whether diagnosis of the disease in its prodromal stage might not be assisted by an agglutination

technique, in which stock emulsions of the Type bacilli were exposed to patients' sera.

(*f*) The relationship which concomitant infection with certain of the commoner anaërobes—other than *B. tetani*—might bear to the causation of tetanus, was also made the subject of inquiry.

SECTION I.

TYPES OF *B. TETANI* OBTAINED FROM WOUNDS OF MEN SUFFERING FROM TETANUS.

This Section of the investigation was undertaken with a view to determining what influence, if any, universally applied serum prophylaxis exerted upon the incidence, course, and issue of the disease, in respect of the serological Types of the bacillus responsible for its causation.

From examination of cultures, seven in number, obtained from various Serum Institutes, it was found that all seven specimens conformed serologically to the "U.S.A. standard culture." As these seven cultures comprised those commonly in use in the English Serum Institutes for the preparation of tetanus antitoxin, it seemed not improbable, that serum, corresponding to one serological type of the bacillus only, was being employed for prophylaxis, so far as the bulk of our own troops was concerned. This might result in the elimination of the disease produced by one serological Type of the bacillus, but might not, to the same extent, reduce the incidence of the disease, when the causal organism was *heterologous* to the prophylactic serum.

This appeared to be strikingly borne out by the examination of the first 25 strains of *B. tetani* obtained from cases of the disease, as typed by the agglutination method. Only one belonged to the same serological group as the standard U.S.A. culture—hereafter referred to as "Type I." At the same time, of the four cultures of toxic tetanus bacilli that had been then isolated from wounds of men not suffering from the disease, three conformed to that Type on serological investigation.

It will be seen from the further consideration of this Section of the Report, that although the hypothesis tentatively advanced above is possibly correct, the demarcation between cases due to Type I bacilli and cases due to the other Types among inoculated men, is not so clear cut as the earlier inquiries might have led one to believe.

(*a*) *Fallacies of investigation.*

In making this investigation, the number of fallacies which may be introduced is such, that any figures obtained and quoted can only have a relative value. Apart from the technical difficulties which have to be overcome in attempting to obtain the organism in a sufficient state of purity to permit of the application of serological tests, other quite uncontrolled sources of error

affect the problem. Great difficulty is, therefore, experienced in interpreting the results obtained.

The present series of cases, 100 in number, represents the successful results obtained in the examination of about 200 specimens. It may seem that a 50 per cent. rate of successful investigations is low, but it must be borne in mind, that the material was not collected by the bacteriologist, and that it was forwarded to the laboratory often from long distances. The investigator was therefore not in a position to obtain optimum results.

Among the cases in which I failed to obtain suitable growths for agglutination, a small number of swabs grew no organisms at all; a larger number grew only aërobes, and in a still larger number there were no organisms resembling *B. tetani*, either in meat-water cultures or in cultures made in media of a more complex composition. A considerable number of the cultures which did develop bacilli bearing a spherical terminal spore, failed to agglutinate in presence of any of the four Type sera. The majority of these agglutinated in presence of a serum prepared by inoculating an animal with a culture of a non-toxic round end-sporing bacillus, whose precise position and relationship have not yet been determined.

In point of fact, it is remarkable, in view of the technical difficulties that are encountered, that not so few, but so many as 50 per cent. were successfully investigated. This purely technical difficulty then, introduces an experimental error, the assessment of which is by no means easy. This error can only be reduced by examination of a further series of cases. In addition to the difficulty of isolation, the occurrence in the cultures of a peculiar inagglutinable phase renders statistical inquiry, based on agglutination, a laborious and not completely satisfactory procedure.

I wish here to emphasise a point that might otherwise be misunderstood, viz., that I do not consider it probable that all organisms capable of elaborating spasm-producing toxins, will necessarily be agglutinated by one or other of the four Type sera; I do think it probable, however, that the majority of such organisms can be so agglutinated.

In any series of cases of tetanus, the incidence, course, and termination of the disease are all modified by a number of factors. These factors, which in each individual case will modify the disease, are:

(i) The nature of the wound, the extent of the solution of continuity, the involvement of muscle or other structures, the degree of devitalisation of tissue due to direct trauma, the degree of interference with the nutrition of the part due to concomitant trauma to vessels, etc.; all these must play a part, and an important part, in the pathogenesis and final issue of any case. Short of visiting and personally examining every case, the part played by each of these factors in the causation of the disease cannot be even remotely estimated.

(ii) The position of the wound and its anatomical relationship may have some bearing on the incidence and result of infection with *B. tetani*.

(iii) The nature and extent of concomitant infection with other organisms is an extremely important, and a quite uncontrollable (uncontrollable from the standpoint of the investigator) factor in determining the onset of tetanus. This question is dealt with to some extent in Section V of the Report.

(iv) As a natural corollary of (*a*) and (*c*) *supra*, the nature of the first surgical interference carried out immediately after the reception of the wound, and, to a less extent, the procedure thereafter followed, will influence profoundly the subsequent history of a case in respect of the incidence of all specific infection—in the present instance infection due to gas gangrene bacilli and *B. tetani*.

(v) The conditions under which the wound was received, notably, the length of time the man lay out before he was picked up, will also have a bearing upon the subsequent development of tetanus and other infections.

(vi) Other conditions, which cannot be dismissed without consideration in this connection, are meteorological, geographical, geological, seasonal and agricultural conditions.

With so many potential sources of error intrinsic to the cases and inseparable from the technique that one is forced to adopt, the present series of 100 cases cannot be expected to give unequivocal information on the point of issue. None the less, the series is of interest and suggests certain not unimportant considerations.

(*b*) *Tabulation of cases due to various types.*

Table I shows the number of cases, the incubation period, and the final result in those instances, in which Type I bacilli were recovered from wound exudates of cases of declared tetanus.

Table II shows the same with regard to Type II bacilli.

Table III shows the same with regard to Type III bacilli.

Table IV shows the same with regard to Type IV bacilli.

Table I. *Cases of Tetanus due to Type I.*

Recovery or death	Prophylactic	Therapeutic	Local	General	Where wounded	Onset
1. Recovered	+	+	...	+	St Quentin ...	7 days
2. Recovered	+	+	...	+	Delville Wood ...	7 „
3. *Died*	+	+	...	+	Vieux Berquin ...	7 „
4. *Died*	−	+	...	+	Peronne	8 „
5. Recovered	+	+	...	+	... ...	9 „
6. *Died*	−	+	...	+	Hull Docks ...	9 „
7. Recovered	+	+	...	+	France	9 „
8. Recovered	+	+	...	+	Delville Wood ...	10 „
9. *Died*	+	+	...	+	Near Maillet ...	10 „
10. Recovered	+	+	+	...	Posières	11 „
11. *Died*	+	+	...	+	Somme	11 „
12. Recovered	+	+	+	...	Bon Hamel ...	11 „
13. *Died*	+	−	+	...	Amiens	12 „
14. Recovered	+	+	+	...	Thiepval	13 „
15. Recovered	+	+	...	+	Somme	13 „

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Table I. *Cases of Tetanus due to Type I—(contd.)*

	Recovery or death	Prophylactic	Therapeutic	Local	General	Where wounded	Onset
16.	Recovered	+	+	...	+	Ypres	13 days
17.	Recovered	+	+	...	+	Mametz Wood ...	14 ,,
18.	Recovered	+	+	+	...	Corbie	16 ,,
19.	*Died*	+	+	...	+	Thiepval	16 ,,
20.	*Died*	+	+	...	+	Cambrai	17 ,,
21.	Recovered	+	+	+	...	La Boiselle ...	18 ,,
22.	Recovered	+	+	...	+	Le Transloy ...	19 ,,
23.	Recovered	+	+	...	+	Bapaume	19 ,,
24.	Recovered	+	+	...	+	Bullecourt ...	19 ,,
25.	Recovered	+	+	...	+	Somme	20 ,,
26.	Recovered	+	+	...	+	Roye	21 ,,
27.	Recovered	+	+	...	+	Bazantin le petit...	21 ,,
28.	Recovered	+	+	...	+	Gouzicourt ...	22 ,,
29.	Recovered	+	+	...	+	Hardicourt ...	29 ,,
30.	*Died*	–	+	...	+	Armentières ...	33 ,,
31.	Recovered	+	+	...	+	Albert	33 ,,
32.	Recovered	+	+	...	+	Ypres	46 ,,
33.	Recovered	+	+	...	+	Neuve Chapelle ...	54 ,,
34.	Recovered	+	+	...	+	Ypres	54 ,,
35.	Recovered	+	+	...	+	... ...	56 ,,
36.	Recovered	+	+	...	+	Somme	58 ,,
37.	Recovered	+	+	...	+	Fricourt	75 ,,
38.	Recovered	+	+	...	+	Ypres	94 ,,
39.	Recovered	+	+	...	+	Somme	101 ,,
40.	Recovered	+	+	...	+	Poelcapelle ...	110 ,,
41.	Recovered	+	+	...	+	Polygon Wood ...	114 ,,

Table II. *Cases of Tetanus due to Type II.*

	Recovery or death	Prophylactic	Therapeutic	Local	General	Where wounded	Onset
1.	Recovered	–	+	...	+	Newton Hungerford	7 days
2.	Recovered	+	+	+	...	Hollebeke	8 ,,
3.	Recovered	+	+	...	+	... ...	8 ,,
4.	*Died*	+	+	...	+	Vimy Ridge ...	9 ,,
5.	Recovered	+	+	+	...	Ypres	10 ,,
6.	*Died*	+	+	...	+	Hirnville	10 ,,
7.	*Died*	+	+	...	+	... ...	10 ,,
8.	Recovered	+	+	+	...	Ypres	12 ,,
9.	Recovered	+	+	...	+	Poelcapelle ...	12 ,,
10.	*Died*	–	+	...	+	France	14 ,,
11.	Recovered	+	+	+	...	Zonnebeke ...	15 ,,
12.	Recovered	+	+	...	+	Guinchy	16 ,,
13.	*Died*	+	+	...	+	... ...	18 ,,
14.	*Died*	+	+	...	+	France	19 ,,
15.	*Died*	–	+	...	+	Abergele (Wales)...	19 ,,
16.	*Died*	+	+	+	...	Ypres	21 ,,
17.	Recovered	– ?	+	+	...	Posières	28 ,,
18.	Recovered	+	+	+	...	Ypres	76 ,,
19.	Recovered	+	+	...	+	Langemarke ...	77 ,,
20.	Recovered	+	+	...	+	Vimy Ridge ...	120 ,,
21. 22.	Details not available.						

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Table III. *Cases of Tetanus due to Type III.*

	Recovery or death	Prophylactic	Therapeutic	Local	General	Where wounded	Onset
1.	*Died*	+	+	...	+	Vimy Ridge ...	3 days
2.	*Died*	+	+	...	+	France	5 „
3.	*Died*	+	+	...	+	Cambrai	6 „
4.	Recovered	+	+	...	+	Villers Bretonneux	6 „
5.	*Died*	+	+	...	+	Thiepval	7 „
6.	*Died*	−	+	...	+	Cambrai	8 „
7.	Recovered	+	+	...	+	Albert	8 „
8.	*Died*	−	+	...	+	Yorkshire	8 „
9.	Recovered	+	+	...	+	Bullecourt ...	9 „
10.	*Died*	+	+	...	+	... ...	10 „
11.	Recovered	+	+	+	...	Amiens	10 „
12.	Recovered	+	+	+	...	Ypres	11 „
13.	*Died*	+	+	...	+	Borsinghe	12 „
14.	Recovered	+	+	...	+	... ...	13 „
15.	*Died**	+	+	...	+	Villers Bretonneux	14 „
16.	Recovered	+	+	...	+	Bullecourt ...	14 „
17.	*Died*	+	+	...	+	Trones Wood ...	14 „
18.	Recovered	+	+	...	+	Trones Wood ...	15 „
19.	*Died*	+	+	...	+	St Quentin ...	16 „
20.	Recovered	+	+	...	+	Passchendaele ...	17 „
21.	Recovered	+	+	+	...	Passchendaele ...	18 „
22.	Recovered	+	+	...	+	Ypres	18 „
23.	*Died*	+	+	...	+	... ...	19 „
24.	Recovered	+	+	...	+	Amiens	20 „
25.	*Died*	+	+	...	+	Somme	30 „
26.	Recovered	+	+	...	+	Gonnelieu	30 „
27.	*Died*	+	+	...	+	Guinchy	43 „
28.	Recovered	+	+	...	+	Blighty Wood ...	54 „
29.	Recovered	+	+	...	+	Albert	57 „
30.	Recovered	+	+	...	+	Guyencourt ...	65 „
31.	Recovered	+	+	...	+	France	81 „
32.	Recovered	+	+	...	+	Cambrai	101 „
33.	*Died*	+	+	...	+	... ...	149 „

* Prophylactic inoculation made late.

Table IV. *Cases of Tetanus due to Type IV.*

	Recovery or death	Prophylactic	Therapeutic	Local	General	Where wounded	Onset
1.	Recovered	−	+	+	...	Posières	4 days
2.	Recovered	+	+	...	+	Villers Pouchée ...	17 „
3.	Recovered	+	+	...	+	Bapaume	48 „
4.	Recovered	+	+	...	+	Ypres	49 „

The findings described in Tables I, II, III, and IV, are graphically summarised in the following Diagram I (p. 112).

Commenting on these results, I wish to call particular attention to a point of great importance, viz., that the prophylactic administration of A.T.S., although failing to prevent absolutely the occurrence of tetanus, tends very markedly to reduce the death-rate from the disease. This is indicated by the fact that death occurred in six out of seven cases of generalised tetanus in

the present series, which did *not* receive a prophylactic dose of A.T.S. Whereas of 75 cases, which were definitely known to have received a prophylactic dose of serum, only 25 terminated fatally.

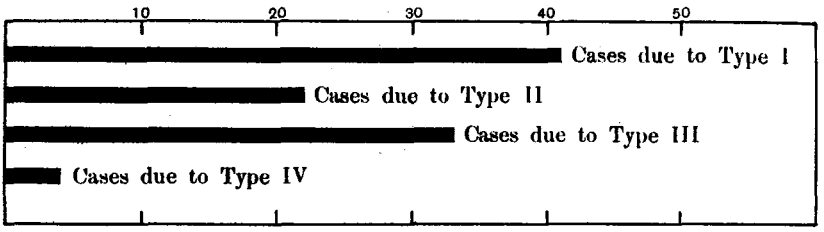

DIAGRAM I. Types of *B. tetani* obtained from 100 cases of tetanus in which the organism was cultured in a sufficient state of purity to permit of serological examination.

If now, we limit ourselves to those cases which received a prophylactic injection of serum the following figures are obtained.

(*a*) Type I appeared to be the organism responsible for the disease in 38 cases; six deaths occurred in this series, of which four were definitely attributable to tetanus, one was certainly not due to that disease, and in the remaining case the cause of death was doubtful. This gives a death-rate of 13·1 per cent. for cases due to Type I.

(*b*) Type II was found in 18 cases, six of which terminated fatally. In five instances death was definitely attributable to tetanus—a death-rate for Type II of 27·7 per cent.

(*c*) Of Type III there were 31 cases with 12 deaths, all of which were attributable to tetanus. In one case the prophylactic dose of A.T.S. was known to have been administered late. Therefore, we may regard the death-rate as 11 out of 31 (= 35·5 per cent.).

(*d*) Of Type IV infection there were only four cases none of which were fatal.

These facts are presented graphically in Diagram II.

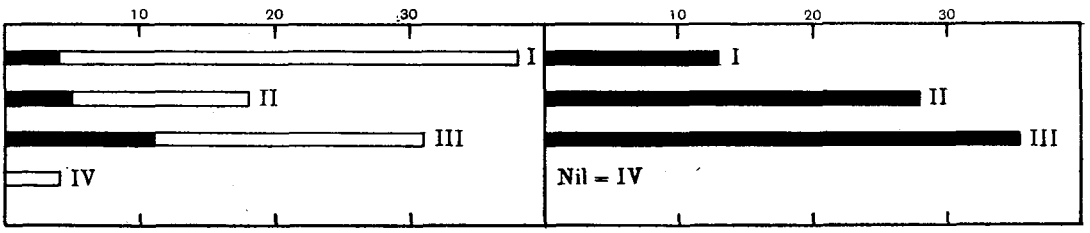

DIAGRAM II. Number of each type of *B. tetani* recovered from declared cases of the disease occurring in inoculated men.

The actual number of deaths due to each type = ■

Deaths from each type of infection expressed as a percentage of the number of cases in which each type was "isolated" from declared cases of tetanus in inoculated men.

These findings call for the following comments.

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(c) *Discussion of findings.*

(*a*) Owing to the total number of cases examined (100) being small, it is difficult to draw definite conclusions from the results obtained. I think, however, that the findings are suggestive, particularly in view of the fact that, both in the case of Type II and in that of Type III infections, the death-rate is considerably higher than when Type I is the organism responsible for the disease.

(*b*) Although Type I bacilli appear to be responsible for a larger number of cases of tetanus than are Types II or III, the death-rate among inoculated men from Type I is lower than when Type II or Type III is the infecting organism.

This might be due simply to there being a relatively larger number of strains of Types II and III virulent to man than there are of Type I; on the contrary, it may mean that the serum used for prophylaxis affords more adequate protection against infection with Type I bacilli than against infection with Types II and III. Further Sections of the present Report deal with attempts which have been made to determine which of these hypotheses is correct.

The figures, so far obtained, are, however, susceptible of inquiry from another point of view. If the low death-rate and (as will be seen from Section III of the present Report) the relatively low rate of incidence of cases due to Type I infection, are attributable to special qualities of the serum used for prophylaxis, then, the number of cases occurring during the first two weeks after wounding, and the proportion of those which terminated fatally, should be relatively greater in infections due to Types II and III than in those due to Type I.

On examining Tables I, II and III, it will be noted that:

1. Of 38 cases of Type I infection in inoculated men the onset of the disease occurred within 14 days in 15 instances, and death was attributable to tetanus in three out of 15 instances.

2. Of 17 cases in inoculated men in which the causal organism was a Type II bacillus, eight occurred within the 14-day period and of these three proved fatal.

3. Of Type III cases there are 31 in inoculated men; in 15 instances the onset occurred within the 14-day period and eight of them proved fatal. Death was attributable to tetanus in all eight cases.

These results may, for purposes of comparison, be graphically expressed thus:

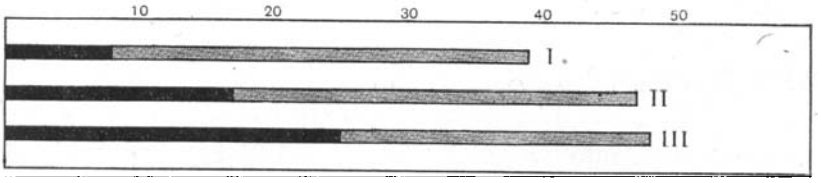

DIAGRAM III. Cases of tetanus in inoculated men due to Types I, II, and III in which onset occurred within 2 weeks. Rates expressed as percentages.

= Incidence. = Death rate.

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Regarded superficially, these figures do not appear to carry conviction, as the numbers dealt with are too small.

A more careful consideration of them, however, indicates that they are extremely suggestive, and a fairly definite interpretation of these figures is made possible by the results obtained in Sections III and V of this Report. Section III shows that Type I bacilli are found much more frequently than are Types II or III in wounds of (inoculated) men who show no evidence of tetanus; while Section V demonstrates that, in passively immunised animals, the development of tetanus infection depends to a preponderating extent upon the degree of tissue destruction occurring at the time of injury. It is only natural then, that Type I cases can and will occur within the 14-day period, and that a certain number of these will prove fatal; for, if the degree of tissue destruction exceeds certain limits, no amount of antitoxin—in practical dosage at least—will prevent the occurrence of the disease.

That the findings obtained in this Section of the work represent a fair average of what would probably be found in examining a larger series of cases is suggested by the fact, that, of about 100 cases of tetanus occurring in inoculated men, from whose wounds I failed to obtain *B. tetani* in a sufficient state of purity to permit of its serological examination, the death-rate was 21 per cent., whereas in the series under discussion, the death-rate is much the same—25 per cent.

General Conclusions from Section I (pp. 107–114).

An examination of 100 cultures of *B. tetani* obtained from wounds of men suffering from tetanus shows:

1. That the value of serum prophylaxis is very great indeed. Practically all the cases of the disease which occur in men who have not received serum prophylaxis terminate fatally.
2. There are indications both from
   (*a*) The higher death-rate, and
   (*b*) The earlier onset of the disease among men who did receive serum prophylaxis, that, either, Types II and III are more virulent for man than is Type I, or, the protection afforded by the serum at present in use, is more adequate against infection due to Type I bacilli than to Types II or III.

SECTION II.

TYPES OF TETANUS BACILLI RECOVERED FROM MEN SHOWING NO EVIDENCE OF TETANUS.

An investigation into the distribution of the various serological Types of *B. tetani* in wounds of men who show no clinical evidence of tetanus is an essential corollary to the previous inquiry. If such an investigation be not made, wrong interpretations might be put upon the findings of Section I,

and a false perspective might be obtained of the actual incidence of the various Types of the bacillus in wounds. During the period in which the series of cases discussed in Section I was being submitted to bacteriological examination, a number of swabs were forwarded to the laboratory from wounds in which organisms resembling *B. tetani* had been demonstrated by other investigators, although the patients showed no evidence of tetanus.

These were submitted to complete bacteriological examination along with cultures containing bacilli resembling *B. tetani* which Miss Cayley encountered in making an investigation into the anaërobic flora of a series of 100 wounds.

In all, 25 such strains were obtained from wounds. These 25 strains were each carefully examined and only qualified as members of one or other of the serological Types of *B. tetani* when they

(1) agglutinated in presence of one or other of the agglutinating sera,

(2) produced toxin when grown *in vitro*, or,

(3) caused tetanus when injected together with a tissue-debilitant.

It may seem peculiar that a differentiation is made between

(*a*) power to produce toxin when grown *in vitro*, and

(*b*) power to produce the disease when washed spores of the culture are injected together with a tissue-debilitant.

But, however, such differentiation of these two factors must be made, in view of the findings which are discussed *in extenso* in Section V of the present Report.

Of these 25 strains which were proved both by agglutination and animal experiment

19 were Type I bacilli
3 ,, ,, II ,,
2 ,, ,, III ,,
1 was ,, IV ,,

The results obtained in Section II are graphically shown in the following diagram—Diagram IV.

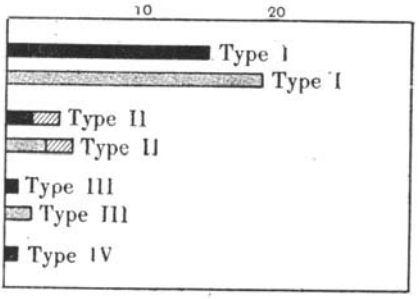

DIAGRAM IV. Actual numbers of *B. tetani*, proved by animal tests and obtained from wounds of men showing no evidence of tetanus.

■ = From series of 100 wounds.
▨ = From all sources.
▧ = Result equivocal.

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The details of the examination of each of these strains are given in the following Table.

Table V.

A. *Strains of* B. tetani *obtained from the examination of* 100 *wounds of men* not *suffering from Tetanus.*

No.	Animal experiment	Agglutination	Since wounding	Where wounded
1.	Toxin expt. positive	Type I	7 days	Ypres
2.	,, ,,	,, I	7 ,,	,,
3.	,, ,,	,, I	20 ,,	Albert
4.	Toxin expt. negative Infection expt. positive	,, I	24 ,,	,,
5.	Toxin expt. positive	,, I	25 ,,	Villers Bretonneux
6.	,, ,,	,, I	30 ,,	Mericourt
7.	Toxin expt. negative Infection expt. positive	,, I	32 ,,	Cambrai
8.	Toxin expt. negative Infection expt. positive	,, I	35 ,,	,,
9.	Toxin expt. negative Infection expt. positive	,, I	39 ,,	,,
10.	Toxin expt. positive	,, I	64 ,,	Battersea
11.	,, ,,	,, I	112 ,,	Ypres
12.	,, ,,	,, I	130 ,,	Cambrai
13.	See Footnote (i)	,, I	733 ,,	Hohenzollern Redoubt
14.	Toxin expt. positive	,, I	860 ,,	Poelcapelle
15.	,, ,,	,, I	882 ,,	Cambrai
16.	,, ,,	,, II	15 ,,	Bapaume
17.	See Footnote (ii)	,, II	100 ,,	Cambrai
18.	Toxin expt. positive	,, II	138 ,,	Passchendaele
19.	See Footnote (ii)	,, II	54 ,,	Monchy
20.	Toxin expt. positive	,, III	16 ,,	Orvillers
21.	,, ,,	,, IV	305 ,,	Hermies

B. *Strains of* B. tetani *obtained from wounds of men* not *suffering from Tetanus obtained from various other sources.*

1.	Toxin expt. positive	Type I	49 days	Cambrai
2.	,, ,,	,, I	56 ,,	,,
3.	,, ,,	,, I	56 ,,	,,
4.	,, ,,	,, I	No details obtainable	
5.	,, ,,	,, II	8 days	No further details
6.	,, ,,	,, III	No details obtainable	

Footnote (i). With reference to No. 13, toxin experiments were negative, and when an attempt was made to carry out the infection experiment, the animals died of gas gangrene.

Footnote (ii). Culture 17 which agglutinated in presence of Type II serum was lost before the animal experiments were completed; culture 19 is still under investigation.

If we limit ourselves to the consideration of those cultures obtained by Miss Cayley in the examination of 100 wounds in Table I A, it is found that

Type I bacilli were found in 15 cases
,, II ,, ,, 2 ,,
,, III ,, ,, 1 case
,, IV ,, ,, 1 ,,

In addition to the two cultures of Type II recorded above there were the two other cases, namely cultures 17 and 19, which could not be fully investigated.

The results dealt with in this Section will be more fully discussed in Section III.

Commenting on these results it is seen:

1. That Type I bacilli appear to be much more frequently found in wounds than are bacilli of the other serological Types.

2. That, in 100 wounds of men who showed no evidence of tetanus, *B. tetani* could be recovered in certainly 19 and probably in 21 instances.

SECTION III.

THE RESULTS OBTAINED IN SECTION I AND SECTION II ARE CONTRASTED.

NOTE ON THE GEOGRAPHICAL DISTRIBUTION OF THE VARIOUS SEROLOGICAL TYPES OF *B. TETANI* ON THE WESTERN FRONT.

If the findings of Section I, which deals with the investigation of swabs from wounds of men suffering from tetanus, be contrasted with the findings of Section II, which deals with the demonstration of tetanus bacilli in the wounds of men showing no evidence of tetanus, the following results are obtained.

The total number of tetanus bacilli isolated from cases of the disease =100.

The total number of tetanus bacilli isolated from wounds of men showing no evidence of tetanus = 25.

Type I from cases	=41	41 %
,, I ,, indifferent wounds	=19	76
,, II ,, cases	=22	22
,, II ,, indifferent wounds	= 3	12
,, III ,, cases	=33	33
,, III ,, indifferent wounds	= 2	8
,, IV ,, cases	= 4	4
,, IV ,, indifferent wounds	= 1	4

This is graphically shown in Diagram V.

Note. In Diagram V, the open column referring to Type II indicates the percentage of proved Type II bacilli obtained from wounds of men not suffering from tetanus.

The hatched column indicates the Type II cultures from the same source in which the investigation is not completed.

These findings also suggest that the protection afforded by the serum at present in use is more adequate in respect of Type I infection than in respect of infections due to Types II and III. The number of Type IV cases is as yet too small to permit of any deductions being made from the results obtained.

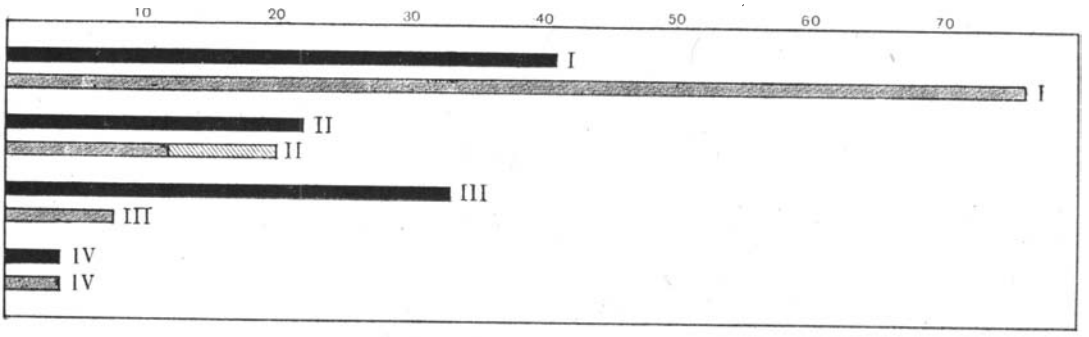

DIAGRAM V. Types of *B. tetani* "isolated" from cases of the disease compared with those "isolated" from "indifferent" wounds each expressed as percentage of the total numbers "isolated" from each source.

= From cases.
= From "indifferent" wounds.
= Type II bacilli from "indifferent" wounds the examination of which was not completed or the result obtained was equivocal.

The figures so far obtained may be dealt with from another standpoint. We know that 20 per cent. of wounds may contain tetanus bacilli, it is also known that the incidence of tetanus among the wounded in England is roughly 1 per 1000. From these figures it appears that in 1000 wounds there are 160 which are infected with Type I bacilli, and therefore, only 1 in 160 infections with that organism gives rise to declared tetanus.

On the same basis 1 in 20 at the lowest, or 1 in 40 at the highest estimate, gives rise to the disease in the case of Type II infections. Of Type III infections there appear to be only 10 per 1000, and therefore, every tenth man infected with Type III bacilli may fall a victim to the disease.

It is questionable if this reasoning is strictly justifiable, for the assumption is made that three cases instead of one have occurred per 1000 wounded men. As the results, however, are only of comparative and not of absolute significance, the same error is introduced in each instance.

The suggestion which arises from the consideration of the facts from this point of view may be diagrammatically shown thus (Diagram VI, p. 119).

The following conclusions appear, therefore, to be permissible from the comparison of the results of the investigations dealt with in Sections I and II.

1. That Type I bacilli are of more frequent occurrence in nature than are Types II and III.

2. That the serum at present in use for prophylaxis affords more adequate protection against Type I infection than against infection with the other Types.

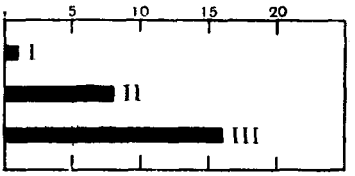

DIAGRAM VI. The columns indicate that of 160 men infected with

Type I bacilli, 1 would take the disease
Type II ,, 8 ,, ,, ,,
Type III ,, 16 ,, ,, ,,

These deductions are made with reserve, as so many factors enter into the causation of the natural disease in man, and so many more appear to affect the toxogenicity of *B. tetani*. Short of unequivocal experimental evidence being obtained, that more adequate protection is afforded by a serum *homologous* to the infecting bacillary Type, than by a serum *heterologous* thereto, one cannot arrive at a definite conclusion concerning the point at issue.

GEOGRAPHICAL DISTRIBUTION OF VARIOUS TYPES OF *B. TETANI* ON THE WESTERN FRONT.

As a matter of scientific interest, if not of practical importance, a record was kept of the districts in France where each man received his injury. In 97 instances this information was available. If the area occupied by British troops be divided roughly into two districts, (1) north of the La Bassée Canal, and (2) south of La Bassée Canal, it is found that:

(i)	of 50 strains of Type I bacilli				13	came from the	N	Area
					37	,, ,,	S	,,
(ii)	,, 15	,,	,,	II ,,	11	,, ,,	N	,,
					4	,, ,,	S	,,
(iii)	,, 27	,,	,,	III ,,	6	,, ,,	N	,,
					21	,, ,,	S	,,
(iv)	,, 5	,,	,,	IV ,,	1	,, ,,	N	,,
					4	,, ,,	S	,,

These figures suggest that the various serological Types may have different geographical distribution in view of the relatively large number of Type II bacilli which were obtained from Flanders. This is further borne out by the fact that during the period of the Flanders offensive Type II were frequently found, while with the alteration of the fighting to the Somme area, Types I and III were those commonly obtained.

It is only fair to note that among a small number of cultures obtained from men who received injuries in this country all types of *B. tetani* were represented.

SECTION IV.

DEMONSTRATION OF ANTIBODIES, OTHER THAN AGGLUTININS, SPECIFIC TO THE SEROLOGICAL TYPES OF *B. TETANI.*

While the previous Sections of this Report suggest that the incidence of tetanus among inoculated men may to some extent depend upon the type of the infecting bacillus, and the serological relationship which it bears to the organism used for the preparation of prophylactic antitoxin, the findings by no means prove that there is such a relationship. In the foregoing Sections stress has been laid upon the difficulty of interpreting the results obtained by making a statistical inquiry into the problem, and these difficulties are again here emphasised.

It was necessary therefore to prosecute experimental investigation while the other phases of the work were proceeding.

A. *The first question which naturally arises is:* "Is the toxin of one serological Type of the bacillus more adequately neutralised by its own *specific* antitoxin than by antitoxin produced by inoculation of the product of a serologically *heterologous* bacillus?"

The findings of Leuchs (*Zeitschrift für Hygiene*, 1918, Bd 65, p. 55) in respect of the toxins of different strains of *B. botulinus* suggested that quantitative differences, at least, might exist between the soluble toxins of the various Types in relation to the neutralising value of an antitoxin corresponding to any one Type.

Experiments were therefore made with a view to examining this point. The results obtained showed definitely:

(*a*) That no *qualitative* difference existed between the toxins produced by the bacillus of the different Types.

One antitoxin neutralised the toxin of any Type or of all Types.

(*b*) That, if a *quantitative* difference exist, it is so slight, that when mice or rats are the animals employed for making these tests, no quantitative relationship of a specific nature can be demonstrated.

B. *The second question which therefore arises is:* "As the soluble toxins of all Types of *B. tetani* are the same, but, as the bacilli themselves are different one from another as *antigens*, is this *antigenic* difference of the bacilli of any import in their pathogenic property, and is it of special importance in the unnatural conditions arising from universally applied serum prophylaxis?"

It seemed not improbable, that the value of antitoxin for prophylactic purposes might depend upon more than one factor:

(1) We know that one factor is the capacity for neutralising the *spasm-producing* toxin of all tetanus bacilli.

(2) Hypothetically, another factor that might be considered is the capacity for preventing *infection.*

The former of these factors we have demonstrated to be non-specific in respect of the Types—at least in so far as the experimental animals employed are concerned. The second (hypothetical) factor, which has not hitherto been inquired into in a systematic way, may be specific. If this be the case, a *mono-typical* serum would protect most adequately against infection with a serologically *homologous* organism.

The inquiry therefore resolved itself into the investigation of the following problems:

(*a*) Can antibodies other than "agglutinins," which are *specific* to the Types, and "anti-spasmins" which are *not specific* to the Types, be evoked by inoculation of cultures of the bacilli or of their products?

(*b*) If such specific antibodies can be demonstrated, and serum containing them be proved to be of prophylactic value, how should we proceed to prepare sera containing these antibodies?

Note. The term "proved to be of prophylactic value" means, proved to prevent *infection* which will cause death in the unprotected animal or in the animal inadequately protected by antitoxin.

(*c*) What relationship do the various demonstrable antibodies bear to one another?

(*d*) What is the mechanism of tetanus *infection* as opposed to tetanus (spasm-producing) *intoxication*?

To investigate these questions two separate and distinct methods of inquiry were followed:

(1) *In vitro* experiments were carried out with various sera and cultures.

(2) Infection experiments *in vivo*.

This Section of the Report (No. IV) deals only with the *in vitro* experiments.

(*a*) *Preparation of sera.*

Nine rabbits were immunised as follows:

I received a course of immunisation with the filtered toxin of a *two-day culture* of Type I bacilli.

The toxin was stored for six weeks in the ice-chest before use, and a *two-day toxin* was chosen so that the least possible autolysis of the bacillus had occurred, and yet an adequate toxin was obtainable.

II was immunised by inoculating intravenously *whole culture* of Type I bacilli grown for 24 hours.

III underwent a course of immunisation with *washed* Type I bacilli.

I′	corresponded to	I	but Type II	*toxin* was used
II′	,,	II	,,	II *culture* was used
III′	,,	III	,,	II *washed bacilli* used
I″	,,	I	,,	III *toxin* was used
II″	,,	II	,,	III *culture* was used
III″	,,	III	,,	III *washed bacilli* used.

The rationale of choosing the three methods of immunisation was that:

(*a*) The animals inoculated with toxin would produce antibodies to the filtrable antigens, and, as stored toxin was used, only antibodies to those antigens which were stable.

(*b*) The animals immunised with all the products of a young culture would produce antibodies to:

(i) any spasm-producing toxin that might have developed,

(ii) to the bacilli themselves,

(iii) to any soluble but unfiltrable or unstable antigens which the organism might develop during its phase of active growth.

It might be argued that it is improbable that a soluble but unfiltrable antigen would be elaborated during the growth of any organism; but if the filtration experiments dealing with the products of pathogenic anaërobes in general be scrutinised, one notes that difficulty has been experienced owing to the large amount of active product that is absorbed in filtration, and filtrates deficient in toxicity have been obtained.

Young cultures were designedly chosen for immunising animals II, II′, II″, because it is known, that certain of the pathogenic anaërobic bacilli—notably *B. Welchii*—elaborate tissue-debilitating poisons during their period of active growth. These poisons are evanescent, and the toxicity of cultures markedly declines on continued incubation for several days at 37° C.

It seemed possible that *B. tetani* might give rise to similar aggressive products that have not so far been demonstrated, because they were not sought for.

(*c*) The animals inoculated with *washed* cultures should produce only antibodies to the bacillary substance.

These nine animals, after having been under immunisation for approximately two months, were bled by Durham's technique to the extent of about 10 c.c. each and a complete series of observations was made with the sera so obtained.

(*b*) *Preliminary investigation of sera.*

Firstly, the antitoxic titre was roughly determined.

Rabbit No. I—approximately 12 units per c.c.
,, II ,, 2 ,, ,,
,, III ,, 1 unit ,,

Note. Animal No. III, owing to an accident, received a number of inoculations with bacilli that had been washed in saline only once, while No. III′ and III″ were immunised with bacilli which had been washed several times.

No. I′ approximately 5 units per c.c.
,, II′ ,, 2 ,, ,,
,, III′ ,, less than 0·5 per c.c.
,, I″ ,, 12 units per c.c.
,, II″ ,, 2 ,, ,,
,, III′ ,, less than 0·5 per c.c.

Secondly, the agglutinin titre of each was determined, and cross experiments with all three Types of bacilli were carried out.

This Section of the work is of considerable importance for two reasons:

(i) A perfectly just criticism might be made of a previous communication on the serological differentiation of tetanus bacilli *inter se* (*Journal of R.A.M.C.*, Dec. 1917), viz. that owing to the low titre of the serum then

Table VI.

	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
	Serum No. I = Type I Toxin						
Type I	–	–	–	–	–	–	–
„ II	–	–	–	–	–	–	–
„ III	–	–	–	–	–	–	–
	Serum No. II = Type I 24-hour Whole Culture						
Type I	++	++	++	++	++	+	–
„ II	–	–	–	–	–	–	–
„ III	–	–	–	–	–	–	–
	Serum No. III = Type I Washed Bacilli						
Type I	++	++	++	++	++	–	–
„ II	–	–	–	–	–	–	–
„ III	–	–	–	–	–	–	–
	Serum No. I′ = Type II Toxin						
Type I	–	–	–	–	–	–	–
„ II	–	–	–	–	–	–	–
„ III	–	–	–	–	–	–	–
	Serum No. II′ = Type II 24-hour Whole Culture						
Type I	–	–	–	–	–	–	–
„ II	++	++	++	++	++	++	–
„ III	–	–	–	–	–	–	–
	Serum No. III′ = Type II Washed Bacilli						
Type I	–	–	–	–	–	–	–
„ II	++	++	++	++	++	++	–
„ III	–	–	–	–	–	–	–
	Serum No. I″ = Type III Toxin						
Type I	–	–	–	–	–	–	–
„ II	–	–	–	–	–	–	–
„ III	–	–	–	–	–	–	–
	Serum No. II″ = Type III 24-hour Whole Culture						
Type I	–	–	–	–	–	–	–
„ II	–	–	–	–	–	–	–
„ III	++	++	++	++	++	–	–
	Serum No. III″ = Type III Washed Bacilli						
Type I	–	–	–	–	–	–	–
„ II	–	–	–	–	–	–	–
„ III	++	++	++	++	++	+	–

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employed—1/400—the specific agglutination results therein figured might give a false impression concerning the complete specificity of the Types of *B. tetani* from the standpoint of agglutination. The low titre of the serum might unduly enhance the specificity of the tests.

(ii) The cultures used for inoculating these animals had been isolated a year previously, and, since isolation, had been frequently sub-cultured—sometimes daily over long periods.

I call attention to this, because it had been suggested that serologically different races of the bacillus might be evolved as a result of residence in human tissue or in close proximity thereto.

Table VI illustrates the agglutination reactions obtained with these sera in presence of emulsions of the Type bacilli.

The results of this titration of agglutinins show that:

(*a*) The *agglutinin* titre is no index of the *antitoxic* value of the serum: this of course is what would naturally be expected.

(*b*) Even when sera of high agglutinin titre are prepared, the agglutination reactions remain *specific.*

(*c*) The stock Type cultures have remained true to Type for a period exceeding 12 months.

(*d*) The agglutinin response is quite as marked when *washed cultures* are used for immunisation as when *whole cultures* are used for this purpose.

Thirdly, the influence which these various sera exhibited in stimulating phagocytosis of Type bacilli was made the subject of inquiry.

(*c*) *Technique of phagocytic tests.*

1. Washed white corpuscles (human) were prepared exactly according to the method advised by Wright; but in place of using a leucocyte layer in making the tests, the deposit of whole blood obtained after the final centrifugalisation was well mixed, and the thick blood cream so obtained was employed.

2. Unwashed actively growing 24-hour cultures served for the bacillary suspension. In order to obtain a sufficiently active growth, these cultures were made in trypsinised broth standardised to be alkaline to α-naphthol-phthalein but acid to phenol-phthalein, and enriched by addition of fresh tissue.

3. The reaction was carried out at 38° C. and incubation proceeded for 15 mins. The mixtures of the various reagents were made and incubated in small agglutination tubes instead of in capillary tubes. This permits of the reagents being well distributed by shaking.

4. Each serum to be examined was tested in a series of dilutions according to the method of Neufeld, and all the sera were fresh when tested.

5. Each mixture consisted of:

1/50 c.c. culture,
1/50 ,, of serum—usually diluted,
2/50 ,, of washed blood cream.

6. After incubation, films were made in the ordinary way, fixed for two minutes in a mixture of equal parts of saturated aqueous solution of $HgCl_2$ and absolute alcohol, and were stained for two minutes with 1/10 carbol-*fuchsin*.

7. Examination of 100 consecutive leucocytes in each preparation was then made. I employed at first a 1/12 objective with a No. 4 ocular for making the counts, but subsequently found that, owing to the size of the bacillus, this could be done quite satisfactorily with 1/6 objective and a No. 4 ocular.

8. The method of Neufeld was adopted, as group relationship between the bacilli might be encountered. Dilution of the serum would to some extent overcome this difficulty.

9. No attempt was made to count the number of organisms taken up by each leucocyte; but, in order to obtain a relative figure, the leucocytes which contained considerable numbers of bacilli were regarded as positive cells, while those containing no bacilli, or only one or two, were considered negative.

Thus, in Table VII and subsequent Tables, the figures given represent the percentage of leucocytes in each preparation which took up the bacilli with avidity. It was remarkable how rarely one met with a cell containing few organisms; the leucocytes were either filled with bacilli or contained none. Difficulty of interpretation did not therefore arise.

(*d*) *Results obtained in making phagocytic tests.*

The results of these phagocytosis experiments are shown in Table II.

Table VII. *The figures relate to the percentage of leucocytes taking up large numbers of bacilli.*

VII. A.

Culture used	No serum control	Serum No. I = Type I toxin. Dilutions of serum 1/20	1/40	1/80
Type I	3	5	6	2
„ II	0	0	0	0
„ III	0	0	0	0
		Serum No. II = Type I 24-hr. whole culture		
Type I	×	85	92	84
„ II	×	10	6	1
„ III	×	77	14	15
		Serum No. III = Type I washed bacilli		
Type I	×	71	48	33
„ II	×	0	0	0
„ III	×	17	9	0

× = Not done.

Note. In this and in subsequent tables relating to phagocytic experiments, the dilutions indicated at the head of each column give the dilution of serum originally made, so that in this instance, the actual dilutions in presence of which the test was carried out were 1/80, 1/160, and 1/320.

VII. B.

Culture used	No serum control	Serum No. I′ = Type II toxin, Dilutions of serum 1/20	1/40	1/80
Type I	1	0	2	2
,, II	0	0	0	0
,, III	0	0	0	0
		Serum No. II′ = Type II 24-hr. whole culture		
Type I	×	1	5	3
,, II	×	68	67	64
,, III	×	0	0	0
		Serum No. III′ = Type II washed bacilli		
Type I	×	5	1	4
,, II	×	50	31	37
,, III	×	0	0	0

VII. C.

Culture used	No serum control	Serum No. I″ = Type III toxin, Dilutions of serum 1/20	1/40	1/80
Type I	0	0	2	1
,, II	0	0	0	0
,, III	2	2	0	0
		Serum No. II″ = Type III 24-hr. whole culture		
Type I	×	87	89	85
,, II	×	28	16	5
,, III	×	99	98	100
		Serum No. III″ = Type III washed bacilli		
Type I	×	2	2	1
,, II	×	4	1	0
,, III	×	44	26	4

Note. Experiments VII A and VII B were done together, using the same reagents, so that they are strictly comparable. My equipment did not permit of the simultaneous examination of all three, so that VII C was done on the following day.

The following points call for comment:

1. The *antitoxic* sera do not stimulate phagocytosis of the bacilli in presence of *whole culture*. It is to be noted, that the observations made with *antitoxic* sera serve as controls for the experiments with *anti-bacterial* sera.

2. The most active sera for stimulating phagocytosis are those prepared by inoculation of *whole culture*.

3. The sera prepared by inoculating *washed bacilli*, while giving an agglutinin titre as high as those prepared by inoculating *whole culture*, do not exhibit so high a phagocytic titre.

4. As regards the question of specificity, sera Nos. III, III′, III″ (*i.e.* antisera to *washed* bacilli) exhibit this markedly in the above experiments, as also does No. II′ (*i.e.* an antiserum to *whole* culture of Type II bacilli),

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whereas Nos. II and II″, also *anti-whole* culture sera to Types I and III respectively, do not.

Note. Animal No. II′ had been under immunisation for a shorter period than II and II″.

In view of the equivocal result obtained with sera Nos. II and II″, the experiment was repeated on the following day, but the dilutions of the sera were carried much further.

Serum No. III, owing to its having a demonstrable antitoxic content, 1 unit per c.c., was also included in this test, the results of which are shown in Table VIII.

Table VIII.

Cultures used	No serum control	Serum No. II = Type I 24-hr. whole culture: Dilutions of serum 1/50	1/100	1/200	1/400	1/800
Type I	0	89	63	50	4	0
„ III	0	5	2	0	0	0
		Serum No. II″ = Type III 24-hr. whole culture				
Type I	×	37	13	4	0	0
„ III	×	98	78	36	0	0
		Serum No. III = Type I washed bacilli				
Type I	×	24	14	2	0	0
„ III	×	1	0	0	0	0

Note. These experiments have been repeated many times and have given consistently specific results on each occasion. The experiment quoted (Table VII A and C) was *that one* of the series, which was most difficult to interpret.

I wish to call attention also to the discrepancy between the actual figures noted in Table VII, and those in Table VIII, in respect of the sera under examination; this appears to depend upon a variability of the anti-phagocytic properties of the cultures, or upon a variability of the phagocytic activity of the white cells from day to day, and renders futile any attempt that might be made to express these results in the form of an index of phagocytosis, unless a standard serum were made the basis of that index.

In order definitely to confirm the most important of the findings obtained in this series of investigations, viz. that *antitoxic* serum did not promote phagocytosis as did *anti-bacterial* serum, the experiment shown in Table IX was carried out.

Table IX.

	1/1	1/10	1/20
	Serum No. I = Type I antitoxic		
Type I	24	0	0
	Serum No. II = Type I 24-hr. whole culture		
Type I	79	82	84
	Serum No. I″ = Type III antitoxic		
Type III	34	0	2
	Serum No. II″ = Type III 24-hr. whole culture		
Type III	70	90	96

Normal serum controls 1/1 were included in the series, and gave substantially the same results as those obtained with 1/1 antitoxic serum.

In making this experiment, the technique already described was followed, but, to ensure that the employment of a 1/6 objective did not lead to avoidable error, the examination of the slides was made with a 1/12 semi-apochromatic lens with a No. 4 ocular, and care was taken critically to illuminate the preparation so that optimum conditions for microscopical examination were realised.

Fourthly, the apparent anti-phagocytic property of whole culture was made the subject of investigation, and an attempt was made to determine whether toxin, as ordinarily prepared and stored, was also anti-phagocytic.

(*e*) *Anti-phagocytic property of whole culture.*

Mechanism of the "anti-phagocytic property" of *whole* culture.

The results quoted in the previous Sub-section of this Report, suggest that the phagocytic activity of leucocytes in presence of anti-serum to *whole* culture may depend upon one or other of two things.

(i) That the serum prepared by inoculation of *whole* culture contains an antibody to an hypothetical aggressive antigen not present in *washed* cultures, and if present in filtrates, only in small quantity, *or*

(ii) That the *bacillary* substance, *per se*, is the antigen that determines the development of the property of stimulating phagocytosis in *anti-bacterial* sera.

It is not improbable that both factors, (i) and (ii), may be represented in the mechanism. By comparing Serum II with Serum III, II′ with III′, and II″ with III″, it is seen, that while the *anti-whole culture* sera are the more active in the presence of *whole* culture, yet the *anti-washed bacilli* sera are not altogether devoid of the power of inducing phagocytosis.

For the present then, leaving out the consideration of the question as to whether these antibodies which lead to the phagocytosis of tetanus bacilli are "stimulins," in the sense implied by Leishman, or "opsonins," in the sense implied by Wright, the point at issue was to determine if:

(*a*) *whole young* culture exhibited aggressive qualities not present in toxin obtained by filtration,

(*b*) these aggressive properties were specific to the serological Types of the bacilli, and could be countered by anti-bacterial serum prepared by inoculating *whole culture*.

The experiments quoted (Tables VII, VIII and IX, pp. 125–127) dispose of the question as to whether these aggressive properties can be neutralised by anti-bacterial (*whole culture*) serum.

To determine whether young *whole* culture did exhibit definite aggressive qualities, and at the same time to determine whether these were or were not specific to the serological Types, the following experiment was set up.

Technique.

Two centrifuge tubes of a 24-hour culture of Type I bacilli and two similar tubes of a 24-hour culture of Type III bacilli were centrifuged at high speed, in order to separate the bacilli from the products of their growth. The supernatant fluid from each tube was drawn off and kept separate. This procedure gave two tubes containing a deposit of Type I bacilli—tubes A and B—and two tubes containing a deposit of Type III bacilli—tubes C and D—and four tubes of supernatant fluid corresponding to these—namely A′ and B′, containing Type I supernatant fluids, and C′ and D′, containing Type III supernatant fluid.

The deposit in each of the four tubes was well shaken with saline and centrifuged. This procedure was repeated twice; after which the saline was finally drawn off with a pipette.

(1) To the deposit of washed Type I bacilli in A was added the supernatant fluid from a Type I culture—A′.

(2) To the deposit of washed Type I bacilli in B was added the supernatant fluid from a Type III culture—D′.

(3) To the deposit of washed Type III bacilli in C was added the supernatant fluid from a Type III culture—C′.

(4) To the deposit of washed Type III bacilli in D was added the supernatant fluid from a Type I culture—B′.

These four suspensions of bacilli were then exposed to washed white cells in presence of dilutions of Type I and Type III *anti-bacterial* sera. The results shown in Table X were obtained.

Table X.

	1/100	1/200
Serum No. II = Type I 24-hr. whole culture		
(*a*) Type I whole culture	75	46
(*b*) Type I bacilli + growth products of Type III ...	72	14
Serum No. II″ = Type III 24-hr. whole culture		
(*c*) Type I whole culture	11	4
(*d*) Type I bacilli + growth products of Type III ...	30	8
Serum No. II = Type I 24-hr. whole culture		
(*e*) Type III whole culture	6	7
(*f*) Type III bacilli + growth products of Type I ...	31	18
Serum No. II″ = Type III 24-hr. whole culture		
(*g*) Type III whole culture	80	67
(*h*) Type III bacilli + growth products of Type I ...	80	38

Note. The ordinary technique was followed in the above experiment, but the observations were made with a 1/12 objective and a No. 4 ocular, in order to exclude technical error as far as possible. In making the count, only those leucocytes containing no organisms were considered negative. This procedure had to be adopted in the present instance, as it was difficult to be sure that centrifugalisation had removed all the bacilli from the supernatant fluid.

Control experiments in which the supernatant fluids alone were exposed to the action of the *homologous* serum in presence of leucocytes showed that:

(*a*) When Type I supernatant fluid + Type I serum 1/100 + blood were incubated, 12 leucocytes were found to have taken up organisms.

(*b*) When Type III supernatant fluid + Type III 1/100 serum + blood were incubated, five leucocytes were found to contain organisms.

The real cause of the low counts obtained was, that there were so few bacilli present that many of the white cells never came into contact with bacilli, and the error therefore, due to the presence in these fluids of organisms that had not been deposited by centrifugalisation, was not very great.

Indeed, the error was more apparent than real, for there were so few bacilli present in these supernatant fluids that white cells containing more than two rods were seldom met with in the controls; while, in the above Table, the majority of the leucocytes considered as positive contained at least four or five bacilli.

In Table X the following points call for comment.

(i) When bacilli, supernatant fluid and serum are all *homologous*, consistently high counts are obtained with both dilutions of the serum—(*a*) and (*g*).

(ii) When bacilli and supernatant fluids are both *heterologous* to the serum, consistently low counts are registered with both dilutions—(*c*) and (*e*).

(iii) Where the bacilli are *homologous* to the serum and the growth products are *heterologous* thereto, a high count is obtained in presence of the higher concentration of the serum; but—as compared with the control, where *homologues* only are present in the mixtures—the lower concentration of the serum shows a relatively low phagocytosis—(*b*) and (*h*).

(iv) When the bacilli are *heterologous* to the serum and the growth products *homologous* to it, a relatively high count—as compared with the control in which both bacilli and growth products are *heterologous*—is obtained with the higher concentration of the serum—(*d*) and (*f*).

(v) The results obtained with *whole* cultures confirm that the reactions are specific to the Types.

From these observations the following deductions seem permissible.

(*a*) That the reaction to inoculation of *bacillary* substance alone, results in the development of antibodies which are specific to each Type and induce phagocytosis.

(*b*) From (iii) it may be deduced that while aggressive substances appear to exist in young cultures, these can, to a considerable extent, be neutralised by an *heterologous anti-bacterial* serum; it is only when the *heterologous serum* is diluted, that the specific aggressive quality becomes manifest.

(*c*) From (iv) the same deduction may be made. For it is seen that leucocytes, even when brought into contact with bacilli which are not *homologous* to the serum present in the mixture, show considerable phagocytic activity, provided that the bacterial products in which the bacilli are sus-

pended *are homologous* to the serum. This activity is naturally most marked in presence of the higher concentrations of serum, and appears to be specific in respect of the products of the various serological Types.

I do not wish to lay over much stress on this Section of the work knowing well the errors, both technical and subjective, that are liable to be introduced in such experiments.

The tests have been repeated and consistent results have been obtained so far as the method allows. Nevertheless, the evidence is by no means unequivocal. I submit, however, that it is highly suggestive, and indicates that there are at least three antigenic factors in cultures of *B. tetani*.

(i) The *spasm-producing toxin*, which is filtrable, and is non-specific in relation to the Types.

(ii) The *bacillary* substance, which is definitely specific to these Types.

(iii) A third *antigen*, which has anti-phagocytic properties, and appears to be specific to the Types.

These findings corroborate those which were obtained when comparative tests were made with *antitoxic*, *anti-whole-culture* and *anti-washed-bacilli* sera; indeed, the deductions which may be made from the one series of experiments are in complete agreement with those that may be made from the other.

Finally, the question of whether toxin, as ordinarily prepared by filtration, exhibits anti-phagocytic properties, was made the subject of investigation.

(*f*) *Examination of toxin to determine whether it is leucotoxic.*

In inquiring into whether tetanus toxin was leucotoxic, I made use of two methods of investigation.

The first series of tests was carried out thus:

Phagocytic tests were made with serum No. III″, which was prepared by inoculation of *washed* Type III bacilli—agglutinin titre 1/3200, antitoxic value < 0·5 unit per c.c., phagocytic titre low. The serum was diluted and exposed to various mixtures of reagents prepared thus:

Five tubes of a 24-hour culture of Type III bacilli were centrifugalised, and the deposit in each was retained. This deposit was washed by adding saline and again centrifugalised, the process being repeated twice. The supernatant saline, after the final centrifugalisation, was pipetted off so that a deposit of washed Type III bacilli was obtained in each of the five tubes—*a*, *b*, *c*, *d* and *e*.

(1) To deposit in (*a*) saline was added to the original volume.

(2) To deposit in (*b*) was added supernatant fluid obtained by centrifuging a 48-hour culture of Type III bacilli.

(3) To deposit in (*c*) was added the supernatant fluid from a 10-day culture of Type III bacilli.

(4) To deposit in (*d*) was added the toxin obtained by filtration of a 48-hour culture of Type III bacilli. This toxin had been stored in the ice-chest for three months before the test was made.

(5) To deposit in (*e*) was added the toxin obtained by filtration of a 10-day culture of Type III bacilli prepared six months before the test was made.

Phagocytic tests were then set up using each of these suspensions as the bacillary emulsions to be phagocytosed. The results shown in Table XI were obtained.

Table XI.

Serum No. III″ = Type III washed bacilli

	1/20	1/40	1/80
Washed bacilli Type III + saline	46	18	9
„ „ + supernatant fluid of 2-day culture	10	2	0
„ „ + „ „ 10 „	3	0	0
„ „ + toxin from 2 „	12	2	1
„ „ + „ „ 10 „	39	13	2

These results show, that both the supernatant fluid and the toxin from the 2-day culture which was used, are definitely anti-phagocytic; while the toxin from the 10-day culture which was included in the test is not so.

It is to be noted that the 2-day toxin was not more lethal for mice than was the 10-day toxin.

With reference to the loss of anti-phagocytic potency in the case of the 10-day toxin, it is probable, that the prolonged storage rather than the prolonged incubation or the filtration, has been responsible for the decline; for, in respect of its anti-phagocytic power, it compares unfavourably both with the 2-day toxin and with the 10-day supernatant fluid.

As the phagocytic potency of Serum III″—*anti-washed-bacillary*—was known to be low, the experiment was repeated, using Serum No. II″—*anti-whole-culture*—the phagocytic potency of which was known to be high, and which also exhibited a demonstrable antitoxic titre. Using this serum the following results were obtained.

Table XII.

Serum No. II″ = Type III 24-hr. whole culture

	1/50	1/100	1/200
Washed bacilli Type III + saline	27	73	61
„ „ + supernatant fluid of 2-day culture	55	45	41
„ „ + „ „ 10 „	64	54	45
„ „ + toxin from 2-day culture	47	44	28
„ „ + „ 10 „	66	65	60

The results shown in Tables XI and XII call for the following comments.

(*a*) The figures in Table XI leave no room for doubt that both supernatant fluids resulting from centrifugalisation of cultures of *B. tetani*, and certain toxins obtained by filtration, are anti-phagocytic. The questions of whether filtration reduces this leucotoxic factor, whether it stands storage, and at what period of growth it is present in largest quantity, or in most active condition, are not answered by the experiment quoted. In this instance —Table XI—it is possible, that owing to the low phagocytic potency of the

serum, and the absence of antitoxic power, a trace of the anti-phagocytic property in the fluids might suffice to obliterate phagocytosis. The conditions of the experiment then do not permit of quantitative results being obtained except under exceptional circumstances, *e.g.* with the 10-day toxin after prolonged storage.

(*b*) The results shown in Table XII, beyond demonstrating that the anti-phagocytic activity both of toxin and of fluid obtained by centrifugalisation are neutralised by *anti-whole-culture* serum, fail to answer the questions raised.

In the second series of experiments an attempt was made to solve certain of these problems, by making indirect experiments, in which an indifferent substance was employed as the body to be taken up by the phagocytes.

It is to be noted, that the results set forth in Tables XIII and XIV are given with every reservation, for subsequent inquiry may lead either to very different results being obtained, or very different interpretations of the facts being made; for the technical difficulties, both subjective and objective, that have to be overcome, are such, that a series of crucial experiments cannot be carried out.

Technique.

The first technical difficulty that arose was the determination of a suitable indifferent substance for phagocytosis. After many trials had been made, I ultimately decided to use a boiled culture of *Staphylococcus albus* for this purpose. The staphylococci, after boiling, were readily taken up by the leucocytes in presence of fresh normal rabbit serum.

Mixtures consisting of 1/50 c.c. of staphylococcus suspension, 1/50 c.c. of the serum under examination, 1/50 c.c. of the toxin to be investigated and 3/50 c.c. of blood suspension were prepared in the same way as for previous experiments. These were incubated for 15 minutes, and preparations made and examined as before. In examining the slides a combination of 1/12 objective and No. 4 ocular was used throughout the series. The results shown in Table XIII were obtained.

Table XIII.

Dilution of serum = 1/2, *i.e.* 1/12 of the final mixtures.

Figures express percentage of leucocytes taking up many staphylococci.

	Serum diluted 1/2				
Toxin (undiluted)	1/50 c.c.	Staphylo. 1/50 c.c.	Blood 3/50 c.c.	Normal serum 1/50 c.c.	Type II antitoxin 1/50 c.c.
No toxin—saline only	...	...	...	69 „	× „
Toxin from 2-day growth Type II	„	„	„	7 „	40 „
„ 10 „ „ II	„	„	„	42 „	72 „
„ 2 „ „ III	„	„	„	31 „	47 „
„ 10 „ „ III	„	„	„	68 „	72 „

The figures indicate that *antitoxic* serum does neutralise the *anti-phagocytic* property of toxin. It may here be noted again, that the toxin from the 2-day culture is more anti-phagocytic than the toxin from the 10-day culture. As to whether the reaction is or is not specific in relation to the Types, the results obtained are equivocal; for the anti-phagocytic power of both the Type III toxins used in the test appears to be less than that of the Type II toxins.

The converse experiment was therefore carried out using a 1/5 dilution of Type III *antitoxic* serum and the results shown in Table XIV were obtained.

Table XIV.

Dilution of serum = 1/5, *i.e.* 1/30 of final mixtures.
Figures express percentage of leucocytes taking up many staphylococci.

Toxin (undiluted)	Serum diluted 1/5							
	1/50 c.c.	Staphylo. 1/50 c.c.	Blood 3/50 c.c.	Normal serum 1/50 c.c.		Type III antitoxin 1/50 c.c.	
No toxin—saline only	...	...	...	86	,,	×	,,
Toxin from 2-day growth Type III	,,	,,	,,	10	,,	63	,,
,, 10 ,, ,, III	,,	,,	,,	41	,,	83	,,
,, 2 ,, ,, II	,,	,,	,,	22	,,	34	,,
,, 10 ,, ,, II	,,	,,	,,	38	,,	83	,,

Note. Experiments XIII and XIV were done on different days so that they are not strictly comparable.

Here again the *anti-phagocytic* property of toxin is apparent, and again, the toxin of the 2-day growth exhibits greater anti-phagocytic power than does that of the 10-day growth. Specificity appears to be fairly definite in this instance, at least so far as the toxin from 2-day growth is concerned. Thus, in the case of the *homologous* mixture, the number of active leucocytes is 63, as contrasted with 10 in the normal serum control. While, in the case of the *heterologous* mixture, it is 34 as compared with 22 for the corresponding control.

The results of these experiments are quite definite in respect of the following points.

(i) Toxin is anti-phagocytic.

(ii) The anti-phagocytic property of toxin can be neutralised by antitoxin.

(iii) If a specific relationship between toxin and antitoxin exist in this connection, the relationship is *quantitative*—the experiments quoted suggest such relationship.

In view of the variability of the anti-phagocytic power of the cultures and their products, a crucial experiment is difficult to perform. Although certain of the results then do suggest such a specific relationship, they fail to demonstrate that relationship unequivocally.

RÉSUMÉ OF SECTION IV.

(I) *Antitoxic sera* do not stimulate phagocytosis of tetanus bacilli.

(II) *Anti-bacterial sera*, prepared by inoculation of *whole* culture, markedly stimulate phagocytosis; and the relationship between serum and organism is specific to the serological Types.

(III) Although *anti-bacterial* sera, prepared by inoculation of *washed* cultures, are active from the standpoint of agglutination, they are not so active in stimulating phagocytosis as are sera obtained by inoculation of *whole* culture.

(IV) The agglutinin titre, the antitoxic titre, and the phagocytic titre of the sera, are independent one of another.

(V) Tetanus toxin is apparently leucotoxic, but all specimens of toxin are not equally leucotoxic.

(VI) This leucotoxic factor can be neutralised by antitoxin; and the experiments quoted suggest that the neutralisation, by antitoxin, of this leucotoxic factor in toxin is specific in relation to the bacillary Types.

SECTION V.

INVESTIGATION OF MECHANISM OF INFECTION IN TETANUS.

INTRODUCTION.

Section IV (pp. 120–135) of this Report shows definitely, that, apart from agglutinins, antibodies specific to the serological Types of *B. tetani* can be evoked by injecting *whole* cultures of that organism into animals. These findings are of considerable significance, but, unless it can be demonstrated *in vivo*, that anti-bacterial immunity plays some part in the prevention or cure of tetanus, their interest is largely academic.

In the first place, it must be clearly understood, that tetanus is due to *the growth* of *B. tetani* within, or in close proximity to, the tissues; and, that intoxication results only when sufficient growth of the organism has occurred. This emphasis of a palpable platitude is necessary, for, in the past, the attention of investigators has been so directed to the mechanism of intoxication in tetanus, that the conditions, which in nature must necessarily precede that intoxication, have been largely overlooked.

In this Section of the Report it is proposed to inquire into the mechanism of *Tetanus infection*, and to determine, if possible, whether the prophylaxis of the disease could be improved in any direction. This really involves the consideration of

(i) Surgical measures.

(ii) Concomitant infections, and how to deal with them.

(iii) The possible value of anti-bacterial sera.

Before these problems could be examined, much preliminary work had to be done in order to elaborate a satisfactory technique for infecting the animals which would be used in the investigation. This presents considerable difficulty, for infection with *B. tetani*, and indeed, infection with any of the pathogenic anaërobes, is, at least in the early stage, saprophytic rather than parasitic in quality. Thus, it has long been known that inoculation of *washed* and *heated* tetanus spores does not frequently lead to the development of the disease, and it is only when a certain amount of tissue destruction has occurred that infection will "take." The same is true of infection with *Vibrion septique*; Besson has shown that spores of this organism may be injected into animals with no ill-effect; if, however, these spores be suspended in a fluid which has leucotoxic properties, infection invariably occurs. Emulsions of *B. Welchii* deprived of their growth products have also been injected without leading to infection, but when injections of the emulsions together with their growth products are made "trauma"—really due to presence of toxin—occurs, leading to the development of the organism.

These considerations have a direct bearing upon the causation of tetanus; for, concomitant infection with any pathogenic organism, by exerting a tissue-debilitating influence, may permit of the development of *B. tetani*. In natural infection of wounds with anaërobes, one cannot consider one infection alone, as every organism in the flora of the wound may exert an influence, stimulant or deterrent, upon the development of any one type of organism present. Wounds probably pass through a cycle of infections; certain aërobes and then certain of the more rapidly growing anaërobes appear in the first phases, to be followed subsequently by the more slowly developing bacteria including *B. tetani*. It must not be assumed, however, that all wounds will pass through the same cycle; for it will depend partly upon the relative numbers of the various organisms present in the infecting material, and partly upon peculiarities of the species or individual exposed to infection, which of the organisms present in the *inoculum* will develop first, and what sequence they will thereafter follow.

In the causation of tetanus the primary *necessary* factor is a certain degree of tissue destruction. In the nidus so formed, organisms grow, and among the earliest usually to appear are certain of the bacilli responsible for the causation of gas gangrene.

These organisms grow rapidly—especially *B. Welchii*—developing growth products which exert a necrotic action upon the tissues. This permits of the further growth of *B. tetani* and of other bacteria of specific infections. A vicious cycle is thus established, and the fact must not be lost sight of, that before even these adjuvant infections occur, it is essential that there should be tissue debility caused by direct trauma to the part, or trauma to important structures in relation thereto.

Therefore, the most important prophylactic measure which can be applied

for the limitation of anaërobic infections is, *adequate and enlightened surgical interference*, performed at the earliest possible moment after reception of the injury. Therefore wide resection of wounds, by removing devitalised tissue, prevents the establishment of this vicious circle.

This question, which is of paramount importance in the experimental work dealt with in this Section of the present communication, is more fully considered in Section VI, pp. 172–195.

These considerations prompted the following questions.

(*a*) DOES *B. TETANI*, IN ADDITION TO ELABORATING A SPASM-PRODUCING SUBSTANCE, ALSO GIVE RISE TO A TISSUE DEBILITATING POISON?

Section No. IV of this Report indicates that *B. tetani* gives rise to tissue-debilitating poisons, for the products of its growth are definitely leucotoxic. In order further to inquire into this problem the following *in vivo* experiment was carried out.

The M.L.D. for rats, of a tetanus toxin obtained from a 10-day growth of bacilli—stored for three months in the ice-chest before use—was first determined. The fraction of the M.L.D. which would invariably give rise to local tetanus but would not cause death was next determined. Mixtures of this quantity of toxin with 2000 million washed but unheated tetanus bacilli (obtained by successive centrifugalisation of rapidly growing cultures suspended in saline) were then made.

(*a*) Mixture consisting of "local tetanus producing dose" of toxin + Type I bacilli 2000 million.

(*b*) Mixture consisting of "local tetanus producing dose" of toxin + Type II bacilli 2000 million.

(*c*) Mixture consisting of "local tetanus producing dose" of toxin + Type III bacilli 2000 million.

(*d*) Saline + Type I bacilli 2000 million.

Four rats were then inoculated, each with one of the above mixtures. All excepting (*d*), which remained well, developed local tetanus between the third and sixth days after inoculation; the spasm continued for 7–10 days and thereafter the animals recovered.

This experiment, while failing to prove, or even to suggest, that *B. tetani* does not develop a tissue debilitating substance, shows that a dose of toxin sufficient to cause a temporary disturbance of the anterior horn cells, may not necessarily exhibit, *locally*, qualities sufficiently aggressive to set up infection.

The *spasm-producing* element of the toxin then is not necessarily a determining factor in the mechanism of *infection*.

I have attempted to carry out the converse experiment but have so far met with no success, *i.e.* I have not been able to obtain a culture possessing sufficient aggressive quality, and at the same time of sufficiently

low "spasmin" content, to permit of a differentiation between local debilitating effect with subsequent *infection*, and immediate disturbance due to central nervous system *intoxication*.

(*b*) AS TISSUE DEBILITATING INFLUENCES CERTAINLY ASSIST IN THE DEVELOPMENT OF TETANUS INFECTION, IS THERE ANY PARTICULAR DEBILITATING INFLUENCE THAT IS OF SPECIAL SIGNIFICANCE IN THIS CONNECTION?

Apart from purely physical considerations such as situation and degree of original trauma, coincident involvement of particular structures, muscle, nerves, vessels, etc., the necrotising influence of the products of certain organisms may play an important part in determining the genesis and evolution of tetanus infection.

That concomitant infection with other organisms might exert such an influence upon the growth of *B. tetani* in wounds, and that this influence might vary with the nature of such concomitant infection, was foreseen by Vaillard and Vincent. The question became insistent when the importance of gas gangrene infections was appreciated in this connection. The following experiments were undertaken with a view to inquiring into the relationship which infection of wounds with *B. Welchii* and *Vibrion septique* might bear to the causation of tetanus.

A preliminary series of experiments was undertaken.

(1) To determine approximately how many washed and heated spores of *B. tetani* could be injected intramuscularly into the guinea-pig without causing the disease.

(2) To determine what dosage of the toxins of *B. Welchii* and *Vibrion septique*, respectively, produced tetanus when injected along with a constant number of spores of *B. tetani*.

In the experiments with *B. Welchii* it was found that 0·2 c.c. of a toxin (which in a dose of 1 c.c. killed one out of three guinea-pigs of 250 grammes weight) sufficed to cause enough local disturbance to determine invariably the onset of tetanus, when injected along with 1000 million spores.

A. Influence of Toxin of *B. Welchii* on the Development of Tetanus Spores *in vivo*.

Experiment I.

Guinea-pig I was inoculated in the right gastrocnemius with 1000 million spores Type I (U.S.A.) in 1 c.c. saline. For the first five days the animal was well; on the sixth and seventh days it limped slightly (? local tetanus); on the eighth day there was no obvious limp, and for the remaining six days during which the animal was under observation it remained well.

Guinea-pig II was inoculated with 1000 million spores Type I plus 0·2 c.c. *B. Welchii* toxin (volume 1 c.c.). The following day there were signs of local tetanus; on the second day there was definite local tetanus; and on the third day the animal died from generalised tetanus.

Guinea-pig III also received 1000 million spores Type I plus 0·2 c.c. *B. Welchii* toxin (volume 1 c.c.) in the right gastrocnemius. One hour later 1400 units of *B. Welchii* antitoxin were injected into the left gastrocnemius. The animal showed no signs of tetanus, and remained well during the period in which it was under observation.

Experiment II.

Guinea-pig I A was inoculated in the right gastrocnemius with 1000 million Type II tetanus spores in 1 c.c. saline. It remained well for the first five days; on the sixth there was a slight limp (? local tetanus); on the seventh day there was definite local tetanus; on the eighth general tetanus; then the animal was killed.

Guinea-pig II A was inoculated with 1000 million Type II tetanus spores, plus 0·2 c.c. B. Welchii toxin (volume 1 c.c.). On the first day the animal appeared to be well; on the second it was moribund, and was then killed.

Guinea-pig III A received 1000 million Type II tetanus spores plus 0·2 c.c. *B. Welchii* toxin (volume 1 c.c.) in the right gastrocnemius. Two hours later 1400 units of *B. Welchii* antitoxin were injected into the left gastrocnemius. For the first five days the animal was well; on the sixth and seventh days it slightly limped (? local tetanus); on the eighth day the limp was less marked; and on the ninth day the animal was well, and it remained well during the period in which it was under observation.

Experiment III.

Guinea-pig I B was inoculated with 1000 million Type III tetanus spores in 1 c.c. saline, in the right gastrocnemius. Guinea-pig II B received 1000 million spores Type III, plus 0·2 c.c. *B. Welchii* toxin (volume 1 c.c.). Guinea-pig III B was inoculated in the right gastrocnemius with 1000 million spores Type III, plus 0·2 c.c. *B. Welchii* toxin (volume 1 c.c.), and received 1400 units *B. Welchii* antitoxin in the left gastrocnemius. Guinea-pig I B remained well; Guinea-pig II B died of tetanus on the third day; and Guinea-pig III B, beyond exhibiting slight stiffness of the inoculated limb from the sixth to the fourteenth day after inoculation, remained well.

The deductions to be drawn from these three experiments are self-evident. They indicate that an antitoxin for *B. Welchii* and tetanus antitoxin should be employed together for the routine serum prophylaxis of tetanus. The evidence is unequivocal that the antitoxin of *B. Welchii*, in addition to neutralising its toxin, has the advantage that it completely protects (in the case of the guinea-pig) tissue which has been exposed to the devitalising effect of that toxin against the development of tetanus spores. It is highly probable that the same protecting value would be found in the case of man.

Attention is called to the death of the control animal I A in Experiment II. The death of this animal, while in no way detracting from the validity of the experiments or of the deductions to be drawn from them, indicates that the development of tetanus depends upon a number of different factors. It is not improbable that, in this instance, a certain degree of trauma when the inoculation was made, was responsible for the development of the infection. It is to be specially noted, however, that while the test animal II A died on the second day after inoculation, the control animal did not die until the eighth day. Moreover the test animal died of acute, almost fulminating tetanus, while the course of the disease in the control animal was much less acute. Experiment II is, therefore, exceptionally instructive; for, while it

shows that the danger of infection with *B. tetani*, and notably the early development of the disease owing to "symbiosis" with *B. Welchii*, could be considerably mitigated—if not completely eliminated—by the use of a combined serum for prophylaxis, it also called attention to the fact, that while such a reagent would probably be of great value, surgical measures should not be less carefully applied because of its introduction.

I call attention to this because, if a combined prophylactic serum were to be introduced, it might lead to the development of a feeling of false security, and too optimistic a view might be taken regarding the probable value of such a combined serum. As a natural sequence its unmerited condemnation would ensue, if the results obtained did not completely bear out expectations.

B. Influence of the Toxin of *Vibrion septique* on the Development of Tetanus Spores *in vivo*.

Vibrion septique is another of the organisms which are commonly found in wounds, and develops diffusible toxic products. While it is not known whether the toxin of this organism is a particularly active necrotising substance or only has marked oedema-producing properties, it seemed of importance, nevertheless, to determine the possible influence which it might exert upon the development of tetanus.

As the properties of this toxin are at present less well defined than are those of the toxin of *B. Welchii*, the experiments to be described in this section cannot be so clearly interpreted as are Experiments I, II and III, pp. 138–139. This difficulty is enhanced by the fact that, at the time of writing, specific *Vibrion septique* antitoxic serum was not available, so that protection experiments could not be undertaken.

I here wish to record my thanks to Miss Robertson of the Lister Institute of Preventive Medicine for placing at my disposal a quantity of the toxin of *Vibrion septique*.

The methods employed and the results obtained in making this investigation were as follows:

Experiment IV.

Guinea-pig A was inoculated in the right gastrocnemius with 0·25 c.c. *Vibrion septique* toxin plus 0·75 c.c. saline. On the first day there was slight oedema and stiffness of the leg. On the second day the oedema was palpable, but not extensive; there was still stiffness of leg. On the third day the oedema was almost gone and the leg less stiff. On the fourth day the animal had almost recovered, and on the fifth day it was well, and remained so during the period of the experiment.

Guinea-pig B was inoculated with 0·25 c.c. *Vibrion septique* toxin, plus 0·25 c.c. saline plus 1000 million tetanus spores Type I. On the first day there was slight oedema and stiffness of leg. On the second day oedema palpable but not extensive; and there was still stiffness of leg. On the third day the animal was recovering, and on the fourth it had almost recovered. On the fifth day it was well, and remained so.

Although the oedema resulting from the injection of 0·25 c.c. of this toxin was quite as marked as, if not more marked than, that produced by 0·2 c.c. of the toxin of *B. Welchii*, it is remarkable that tetanus did not develop. I therefore decided to make the test much more stringent, employing 1 c.c. of the toxin plus 1000 million tetanus spores. The result of an experiment employing this technique is shown below.

Experiment V.

Animal C was inoculated in the right gastrocnemius with 1 c.c. *Vibrion septique* toxin plus 0·5 c.c. saline. On the first day there was marked oedema over the whole of the injected limb; on the second the oedema was more marked and extending over the abdominal wall, soft on palpation. The next day the oedema was reduced and the animal lively; limb stiff. On the fourth day the oedema was disappearing; leg still stiff. On the fifth day the animal had recovered, and remained well till the conclusion of the experiment.

Animal D received 1 c.c. *Vibrion septique* toxin plus 1000 million Type I tetanus spores. On the first day there was marked oedema over the whole of the injected limb; on the second, oedema as in Animal C. On the third day the oedema was reduced and the animal lively, but limb stiff. Fourth day, oedema almost gone; leg still stiff. On the fifth day the animal was well, and remained so.

A slight stiffness of the limb remained in both animals until the completion of the experiment, which was considered to have occurred on the fourteenth day. The animals have remained well, and the stiffness is slowly disappearing. This experiment is striking, in that, although the oedema produced in the animals was much more marked than that resulting from the injection of 0·2 c.c. of the toxin of *B. Welchii* employed in the previous series of experiments, no development of tetanus occurred.

This result was scarcely expected, and the experiment was therefore repeated.

Experiment VI.

Animal E was inoculated with 1000 million Type I tetanus spores suspended in 1·5 c.c. of saline. Animal F with 1 c.c. of *Vibrion septique* toxin plus 1000 million Type I tetanus spores. These animals both remained well, so corroborating the findings indicated in Experiment V.

As the disturbance produced by *Vibrion septique* toxin—oedema production—was so pronounced, it seemed remarkable that tetanus did not develop. The experiment was repeated a second time, using spores of a representative Type III tetanus bacillus. The findings of this experiment are as follows:

Experiment VII.

Animal G was inoculated in the right gastrocnemius with 1000 million Type III tetanus spores suspended in 1·5 c.c. saline. It remained well. Animal H was inoculated with 1000 million Type III tetanus spores plus 1 c.c. *Vibrion septique* (volume 1·5 c.c.). There was marked oedema on the first day, but the animal was active. On the second day there was general tetanus, and the animal was killed.

As specific antitoxin for *Vibrion septique* was not available, it was decided not to complete the present series of experiments using Type II spores; for the results shown in Experiment VII indicate that *Vibrion septique* may, like *B. Welchii*, play an ancillary part in the causation of tetanus.

The negative results obtained on three occasions when Type I spores were used indicate, however, that the toxin of *Vibrion septique* is probably a less constant factor in stimulating the growth of *B. tetani* in the tissues, than is that of *B. Welchii*. In view, however, of its frequent occurrence, and in view of the fact that it sporulates more readily than does *B. Welchii*, its capacity for doing harm is possibly extended over a more prolonged period than is the case with *B. Welchii*. It would seem advisable therefore, that antibodies to the toxin of *Vibrion septique* should also be included in serum used for the prophylaxis of tetanus.

CONCLUSIONS FROM THIS SERIES OF EXPERIMENTS, pp. 138–142.

1. There is good ground for believing that the ancillary part played by *B. Welchii* in the causation of tetanus is clearly defined. The capacity of this organism for doing harm in the connection under consideration can be almost eliminated by the use of the antitoxin for *B. Welchii*.

2. The capacity of the toxin of *Vibrion septique* for stimulating the growth of tetanus spores *in vivo* is more variable than is that of *B. Welchii*. Experiment VII indicates, however, that it too may play a part in the causation of tetanus.

3. It follows from conclusions 1 and 2 that antibodies to the toxins of *B. tetani*, *B. Welchii*, and *Vibrion septique* should be included in all serum employed for the prophylaxis of tetanus.

4. While such a polyvalent serum promises to reduce still further the incidence of tetanus, we may not assume that it would absolutely eliminate that disease, for infections other than those dealt with in this communication may also play a part in stimulating the growth of *B. tetani* in wounds.

(*c*) AS CONCOMITANT INFECTION WITH CERTAIN ORGANISMS STIMULATES THE GROWTH OF SPORES OF *B. TETANI* IN TISSUE, IS IT NOT POSSIBLE THAT THE CONVERSE MAY BE EQUALLY TRUE? AT LEAST, MAY NOT SOME CONCOMITANT INFECTIONS REDUCE THE TOXOGENIC CAPACITY OF *B. TETANI* UNDER CERTAIN CIRCUMSTANCES WHICH ARE AT PRESENT UNKNOWN?

The facts dealt with in this Sub-section of the Report were obtained, not in making inquiry into the above question, but in conducting an investigation with another object in view. This problem is complementary to (*b*), p. 138. In examining wound exudates from men showing no evidence of tetanus, Miss Cayley obtained three consecutive cultures, which failed to develop toxin when tested by the ordinary methods, but nevertheless contained bacilli in large numbers having the morphological characters of *B. tetani* and, on investigation by the method described in a previous com-

munication (*Journal R.A.M.C.*, Dec. 1917), agglutinated in presence of Type I serum. This finding meant that

(*a*) certain strains of *B. tetani* were non-toxogenic, or

(*b*) that certain organisms other than *B. tetani* agglutinate in presence of Type I serum, or

(*c*) that the conditions under which the organisms were growing rendered them temporarily non-toxogenic, or that toxin was destroyed as rapidly as it was produced.

In order to determine by *in vitro* methods whether these cultures contained Type I bacilli or merely other micro-organisms exhibiting marked group agglutination with Type I serum, they were fully examined by microscopical and serological methods.

The three cultures under consideration will be hereinafter designated C 5, C 14 and C 15.

A. The original cultures of wound exudates were made in autoclaved meat broth and their examination showed the following:

(i) C 5 on the 7th day after seeding showed the presence of organisms of the tetanus morphological type, the sporogenes type and oval end-sporing bacilli.

(ii) C 14 showed tetanus type on the 14th day of incubation with sporogenes and oval end-sporing types also present.

(iii) C 15 showed tetanus type on the 9th day of incubation, sporogenes type also being present.

B. Sub-cultures were made from these original meat broth tubes in the enriching medium described in a previous communication (*Journal R.A.M.C.*, Dec. 1917).

(i) C 5 in the enriching medium gave after three days' incubation a strong growth of bacteria having the morphological characters of *B. tetani*.

(ii) C 14 behaved in a similar way, but sporulation was not marked until the 4th day of incubation.

(iii) C 15 behaved exactly as did C 14.

C. These cultures were then centrifugalised, washed in saline, and suspensions made and standardised by the opacity method, to contain 2000 million bacilli per c.c. The suspensions were then heated and phenolated, following the standard technique, and finally exposed to the action of Types I, II, and III, agglutinating sera. The results obtained are shown in Table XV.

Table XV.

Culture	Type I serum			Type II serum			Type III serum			Normal
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400	1/50
C 5	++	++	+	–	–	–	–	–	–	–
C 14	++	++	+	–	–	–	–	–	–	–
C 15	++	++	++	–	–	–	–	–	–	–
Controls	++	++	++	++	++	++	++	++	++	–

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Absorption of agglutinin tests were then carried out again using the standard technique, and results shown in Table XVI were obtained.

Table XVI A.

Absorption of Type I Serum by Cultures C 5, C 14, C 15.

Culture	Unabsorbed serum				Absorbed serum							
	Agglutination				Homologous bacillus added				Test bacillus added			
	1/100	1/200	1/400	1/800	1/100	1/200	1/400	1/800	1/100	1/200	1/400	1/800
C 5	++	++	+	−	++	−	−	−	−	−	−	−
C 14	++	++	+	−	++	−	−	−	−	−	−	−
C 15	++	++	+	−	+	−	−	−	−	−	−	−
Control	++	++	++	−	−	−	−	−	−	−	−	−

Table XVI B.

Absorption of Type II Serum by Cultures C 5, C 14, C 15.

Culture	Unabsorbed serum				Absorbed serum							
	Agglutination				Homologous bacillus added				Test bacillus added			
	1/100	1/200	1/400	1/800	1/100	1/200	1/400	1/800	1/100	1/200	1/400	1/800
C 5	−	−	−	−	++	++	++	+	−	−	−	−
C 14	−	−	−	−	++	++	++	+	−	−	−	−
C 15	−	−	−	−	++	++	++	+	−	−	−	−
Control	++	++	++	+	−	−	−	−	−	−	−	−

Table XVI C.

Absorption of Type III Serum by Cultures C 5, C 14, C 15.

Culture	Unabsorbed serum				Absorbed serum							
	Agglutination				Homologous bacillus added				Test bacillus added			
	1/100	1/200	1/400	1/800	1/100	1/200	1/400	1/800	1/100	1/200	1/400	1/800
C 5	−	−	−	−	++	++	++	+	−	−	−	−
C 14	−	−	−	−	++	++	++	+	−	−	−	−
C 15	−	−	−	−	++	++	++	+	−	−	−	−
Control	++	++	++	+	−	−	−	−	−	−	−	−

The findings shown in Tables XV and XVI indicate that the three cultures under consideration were Type I tetanus bacilli, and, in view of their failure to produce toxin when tested in the ordinary way, inquiry had to be made to determine whether:

(*a*) They were intrinsically atoxic.

(*b*) The circumstances of their growth reduced their toxogenic capacity.

D. Animal experiments were therefore undertaken to investigate these points.

(1) In the first place sub-cultures into ordinary peptone broth were made from the original meat cultures, and were incubated anaërobically for six days.

Microscopical examination of the growths was then made to determine the

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presence of organisms bearing spherical terminal spores. A massive inoculation —0·5 c.c.—of these growths was made intramuscularly into each of three rats. All the animals remained well, so confirming the original experiment. The experiment was repeated with mice and the same result was obtained.

(2) As it seemed possible that the tetanus bacilli might have been introduced into the enriching medium through technical error, sub-cultures were made in broth from the tubes containing growths of C 5, C 14 and C 15 in the enriching medium. After 10 days 0·5 c.c. of each of these sub-cultures was inoculated into mice.

Notwithstanding these massive inoculations two of the mice remained well. That inoculated with C 14 died from tetanus on the 5th day. It seemed improbable then, that the tetanus bacilli had been introduced into the enriching medium during any of the various processes of its preparation. In order further to ensure that the medium was not a source of error, sub-cultures from the original meat tubes were made in three different batches of enriching medium, and agglutination tests were repeated with the cultures so obtained. All gave consistent results.

Note. Before any batch of enriching medium is used in the laboratory, it is examined by cultural methods to ensure sterility, and where animal experiments are to be performed with growths of organisms in this medium, it is inoculated into mice to ascertain that the medium itself does not exhibit toxic properties.

(3) The following experiments were then set up to determine, if possible, whether the tetanus bacilli in C 5, C 14 and C 15 were themselves atoxic, or whether their toxogenic capacity was interfered with by concomitant growth of other bacteria present in the cultures.

(i) Four tubes of meat broth, *a*, *b*, *c*, and *d*, were each inoculated with an equal quantity of the same culture of Type I bacilli—the U.S.A. standard culture which was known to be toxic.

Into Tube "*b*" was then seeded some of the original meat culture of C 5,
„ "*c*" „ „ „ „ C 14,
„ "*d*" „ „ „ „ C 15.

Tube "*a*," which was inoculated with the standard U.S.A. culture only, served as a control.

All four tubes were incubated for eight days, and then 0·25 c.c. of each culture—again a massive inoculation for mice—was injected subcutaneously into mice.

The mouse inoculated with "*a*" died from tetanus within 24 hours; that inoculated with "*b*" showed tetanic symptoms from the 3rd to the 8th day, but eventually recovered; that inoculated with "*c*" remained well; while the animal which received "*d*" developed tetanus only on the 5th day and died from the disease on the 6th.

(ii) The experiment was repeated but the tubes in this instance were incubated for 10 days.

The animal inoculated with "*a*" died within 24 hours; that with "*b*" showed tetanic symptoms from which the animal ultimately recovered—the onset of the condition occurring six days after inoculation. The animals inoculated with "*c*" and "*d*" remained well.

(iii) Exactly similar experiments were then carried out using cultures from mixed *inocula* of Type II and Type III bacilli and C 5, C 14 and C 15. The cultures were incubated for eight days and inoculated as in (i) and (ii). In neither case, however, did any of the growths prove atoxic.

(iv) Experiment (i) was repeated a second time and gave unequivocal confirmation of the previous findings.

(v) As experiments (i) and (ii) of this Sub-section indicated that the toxic qualities of mixed cultures might vary from day to day I made four sub-cultures each of C 5, C 14 and C 15 for inoculation on different days. One series of tests was made after four, one after six, one after eight, and one after ten days' incubation. The *inocula* consisted of 0·5 c.c. in each instance, and the animals used were rats. All the animals in this series of experiments survived, and none showed evidence of tetanus.

(vi) The experiments described in (i), (ii) and (iv) indicate that co-incident growth of other organisms may prevent the development of tetanus toxin in cultures.

In order finally to dispose of the question, sub-cultures from the original meat tubes of C 5, C 14 and C 15, were made in the enriching medium, and the growths so obtained were centrifugalised, washed, and heated, as for the preparation of suspensions for agglutination. In doing this, special precautions were taken to prevent the occurrence of contamination. A specimen of each of the suspensions so obtained was retained for animal inoculation, and the bulk of each suspension was agglutinated. Again all three cultures reacted well in the presence of Type I agglutinating serum.

The specimens removed from each suspension before agglutination were dealt with thus:

(*a*) 0·1 c.c. of suspension of C 5 was mixed with 1/4 M.L.D.—for mice—of *Welchii* toxin. The mixture, so prepared—total volume 0·2 c.c.—was injected subcutaneously into a mouse. The animal developed tetanus and died on the third day.

(*b*) 0·1 c.c. of suspension of C 14 + the same quantity of *Welchii* toxin was injected into a second mouse. The animal developed tetanus, and was killed on the second day after inoculation.

(*c*) An exactly similar experiment in which the suspension of C 15 was tested resulted in the development of tetanus on the third day after inoculation.

(*d*) A control mouse, inoculated with the *Welchii* toxin alone, remained well.

The experiment was repeated using a smaller dose of *Welchii* toxin, and substantially the same result was obtained.

Commenting on these results, it is remarkable that there is such definite evidence of loss of toxogenic power, when the Type I culture is grown in a mixture along with the cultures under consideration. It is also remarkable that a similar result was not obtained with the Type II and Type III bacilli—Experiment (iii), p. 146.

This finding might suggest that cultures of Type I bacilli were more susceptible to depression of their toxogenicity by certain mixtures than were cultures of Type II and III. This is possibly so; but, short of carrying out a long series of quantitative experiments with numerous strains of each Type, such a deduction is not permissible. It is more probable that the particular strain of Type I used was—perhaps only temporarily—less toxogenic than the strains of Types II and III employed, and therefore the conditions necessary for demonstrating depression of their toxogenicity were not realised.

The important point is, as the experiments definitely show, that certain mixtures of organisms depress to a greater or less extent the toxin-producing capacity of certain strains of tetanus bacilli when grown together with them. Experiment (vi), p. 142, of the series shows that spores of tetanus bacilli obtained from such non-toxic mixtures are definitely toxogenic when inoculated into animals under suitable conditions.

(*d*) PRELIMINARY EXPERIMENTS CONDUCTED WITH A VIEW TO THE ELABORATION OF A METHOD FOR STUDYING INFECTION WITH *B. TETANI*.

Investigations conducted for the purpose of determining a suitable tissue debilitant, which might be employed for starting the infective process in various animals.

The experiments already described indicate that a method might be elaborated for studying the various problems of the prophylaxis and therapeutics of tetanus from the standpoint of *infection* instead of from the standpoint of *intoxication*.

Could this be done, a more rational view of the pathogeny of the natural disease would be obtained, and, it is not improbable, that improvements in the methods of prevention, if not in the methods of treatment, might be suggested by the findings obtained.

The primary object of the investigation was to obtain, if possible, a standard method of *infection*. This would constitute a basis for the study of the prophylactic values of *anti-bacterial* and *antitoxic* sera; and also for the study of the action of "mono-typical" sera in order to determine whether they are or are not specific.

The experiments described on p. 142 show that the toxin of *B. Welchii* might be used for this purpose in guinea-pigs and in mice; it also sets up infection in the rat. The employment, however, of a biological reagent of this nature, for the purpose in view, presents considerable difficulties, owing to its instability, and owing also to its somewhat inconstant

effect upon different members of even one animal species, because of the variation in susceptibility exhibited. I have employed it in conducting a series of observations which will be described later, but found it unsatisfactory; because, if reliable results are to be obtained, it is essential that a complete series of experiments be performed at one time. This precluded its use in experiments in which guinea-pigs were employed, owing to the expense incurred, and, as will be seen from subsequent observations, infection experiments are difficult to carry out under standard conditions.

When a standard number of spores together with the debilitant are injected into animals, it is found that a narrow margin exists between the degrees of tissue debility which, on the one hand, will certainly give rise to the development of tetanus and which, on the other hand, will cause death in most animals even though protected by large doses of antitoxin.

By the term "a large dose of antitoxin," I mean such a (relative) quantity as would be quite impracticable as a prophylactic dosage in man; *e.g.* 50 units for a guinea-pig is equal to 12,000 units for man.

A number of substances were therefore investigated in an attempt to obtain a more stable, and therefore more constant irritant than *Welchii toxin*, or any other biological product, and which could moreover be standardised by physical or chemical methods. The substances examined were:

1. *Lactic Acid.* Various solutions of this reagent were tried both on guinea-pigs and mice, which were inoculated at the same time with a standard number of spores. The results were so inconstant that the reagent was obviously unsuitable for the purpose in view.

2. *Trimethylamine.* This reagent was also tried and was found to give fairly constant results in the case of guinea-pigs; but its marked alkalinity and its tendency to undergo decomposition proved it to be a less reliable substance than saponin for the purpose of setting up infection in these animals.

Trimethylamine could not be satisfactorily used in the case of mice, for a somewhat unexpected result was obtained with them, illustrating the difficulty of the problem under consideration, and which I think is worthy of record.

Twelve mice were inoculated subcutaneously with the following mixtures of spores and trimethylamine.

(*a*)	100 million	Type	I	spores	+ 0·1 c.c.	trimethylamine	33 %	+	−		
(*b*)	,,	,,	II	,,	+ ,,	,,	,,	+	−		
(*c*)	,,	,,	III	,,	+ ,,	,,	,,	+	−		
(*a′*)	,,	,,	I	,,	+ 0·05 c.c.	,,	,,	+ 0·05	saline		
(*b′*)	,,	,,	II	,,	+ ,,	,,	,,	+	,,		
(*c′*)	,,	,,	III	,,	+ ,,	,,	,,	+	,,		
(*a″*)	,,	,,	I	,,	+ 0·025 c.c.	,,	,,	+ 0·075	saline		
(*b″*)	,,	,,	II	,,	+ ,,	,,	,,	+	,,		
(*c″*)	,,	,,	III	,,	+ ,,	,,	,,	+	,,		
(*a‴*)	,,	,,	I	,,	+ 0·0125 c.c.	,,	,,	+ 0·0875	saline		
(*b‴*)	,,	,,	II	,,	+ ,,	,,	,,	+	,,		
(*c‴*)	,,	,,	III	,,	+ ,,	,,	,,	+	,,		

The volume of each *inoculum* was 0·3 c.c.

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(1) Animals (*a*), (*b*), and (*c*), were all found dead the morning following the inoculation. They showed no evidence of tetanus.

(2) (*a'*), (*b'*), and (*c'*), each developed an eschar about $\frac{1}{4}$ of an inch in diameter at the site of injection, two days after inoculation. Notwithstanding this, they remained well, were lively, took food greedily, and after a further lapse of three or four days, all but one had recovered. The animal which died—(*b'*)—did not show any evidence of tetanus, and only succumbed after the eschar had completely healed.

(3) (*a''*) developed definite tetanus on the 7th day after inoculation and was killed.

(*b''*) showed demonstrable tetanic spasm on the 3rd day and it, too, was killed.

(*c''*) died from tetanus on the 2nd day.

(4) (*a'''*), (*b'''*), and (*c'''*), all remained well.

From the above experiments it is seen that only the third series of tests gives results which are at all encouraging; but the time variation before the onset of the disease, makes the employment of diluted trimethylamine impracticable for experiments designed to investigate the prophylactic value of sera.

It is very remarkable that those animals which received 0·05 c.c. of the reagent, although they showed marked local disturbance, did not succumb to tetanus; while those which received 0·025 succumbed although they showed practically no local disturbance.

3. *Saponin.* The use of this reagent was suggested to me by Dr F. Ransom and I wish to thank him cordially for his advice.

The following experiments were carried out to determine how this substance might be employed in order to set up infection. It is to be noted, that the great advantage of saponin lies in the fact that its solutions can be standardised by *physical* methods, and it can be sterilised by autoclaving, without losing its irritant properties; solutions should not, however, be autoclaved more than once.

Five guinea-pigs were inoculated intramuscularly with the following mixtures:

Animal	1,	saponin	1/100	0·2 c.c.,	saline	0·2 c.c.,	Type I spores	200	million—Vol. = 0·6 c.c.	
„	2,	„	1/200	„	„	„	200	„	„	„
„	3,	„	1/400	„	„	„	200	„	„	„
„	4,	„	1/800	„	„	„	400	„	„	„
„	5,	„	1/1600	„	„	„	400	„		„

Number 1 showed evidence of local tetanus on the morning of the 2nd day, rapidly developed tetanic convulsions and was therefore killed.

Number 2 had stiffening of the inoculated limb on the evening of the 2nd day and was found suffering from generalised tetanus on the morning of the 3rd day. It was then killed, as the possibility of recovery was remote.

Numbers 3, 4 and 5 remained perfectly well. This experiment gave an indication of how a standard infection might be set up in the guinea-pig—

at least so far as a standard infection of any kind can be set up in any animal. Subsequent experiment showed that 200 million spores + 0·2 c.c. of 1/200 saponin gave consistent results, tetanus invariably developing on the 2nd or 3rd day after the intramuscular injection of the mixture. Equal volumes of 1/300 dilution of the same sample of saponin in presence of the same number of spores did not give constant results.

A similar series of tests was made with mice, dilutions of saponin 1/50–1/400 being tested, but the results obtained were entirely negative. The test was done in triplicate, using spores of all three types of *B. tetani*. The animals remained well when the lower concentrations were used, and showed absolutely no evidence of tetanus, but succumbed to saponin intoxication, usually within 24 hours, when the higher concentrations were injected.

The results of these experiments, dealing with the employment of irritants for setting up infection, show the following interesting points.

(i) *Welchii* toxin, although unsatisfactory for performing quantitative tests, proved to be the most consistent of the reagents investigated, in respect of its power of producing infection in a variety of animal species.

(ii) The chemical reagents are less consistent, *e.g.* saponin sets up infection in the guinea-pig but not in the mouse.

(iii) The dilution of the reagent employed may have a very marked influence, and sometimes an unexpected one, in respect of its power to give rise to infection in different animals. This fact is strikingly illustrated in the case of trimethylamine. When this reagent is used on mice it shows, that not only the degree, but also the nature of the tissue-debilitating lesion may profoundly affect the development of tetanus.

(iv) In the guinea-pig, saponin can be used to set up an infection, and gives fairly constant results. The animals die two to three days after inoculation. A method of testing the *anti-infective* properties of sera in guinea-pigs seems, therefore, to have been obtained.

(v) The experiment, in which the infection-stimulating properties of saponin were investigated, shows that the degree of tissue destruction, rather than the number of spores inoculated, is the determining factor in causing the development of *infection*. Thus animals 4 and 5 did not develop the disease although they received twice the number of spores injected into animals 1, 2 and 3.

(*e*) EXPERIMENTS DEALING WITH PROPHYLAXIS WITH ANTI-TETANIC SERUM.

(i) *Degree of tissue destruction in its relation to the causation of tetanus.*

(ii) *Degree of immunity conferred by varying the dose of antitoxin.*

In the previous Sub-section, pp. 147–150, attention is called to the important bearing which the degree of tissue debility may have upon the

development of tetanus, when a constant number of spores are inoculated. This raises the question: "Does antitoxin, used in doses having a ratio practicable in man, prevent a fatal issue in experimental animals, when the degree of tissue destruction is great?"

In an attempt to inquire into this problem the following experiment was carried out:

Four rats, (*a*), (*b*), (*c*) and (*d*), each weighing approximately 100 grammes, received 40 units of antitoxin; the antitoxin used was mark "B.W. horse 13."

After an interval of two days animal

(*a*) was inoculated with a mixture of *Welchii* toxin + 500 million Type I spores,
(*b*) „ „ „ + 500 „ „ II „
(*c*) „ „ „ + 500 „ „ III „
(*d*) „ „ „ + saline.

The dose of *Welchii* toxin chosen was that which produced a marked oedema of the whole limb when injected by the intramuscular route. Controls which had received no antitoxin were also injected with the same mixtures. The control animals and also animals (*a*), (*b*) and (*c*) all developed tetanus and died, the fatal issue in the experimental animals as compared with the control animals being delayed only for 24 to 48 hours. Animals injected with tetanus spores only remained well. Animal (*d*)—*Welchii* toxin alone—naturally developed a marked oedema of the inoculated limb, but recovered completely in four or five days.

The experiment was repeated on mice, which were given 5 units of antitoxin two days before the inoculation of spores and *Welchii* toxin. An exactly similar result was obtained.

If the dose of antitoxin used in these experiments be expressed as gramme weight equivalents for man the ratio in each instance is:

40 units to a rat of 100 grammes is equal to
24,000 „ „ man of 60 kilos.
5 „ „ mouse of 15 grammes is equal to
20,000 „ „ man of 60 kilos.

The results indicate that the development of infection with *B. tetani*, in passively immunised animals, depends largely upon *quantitative* factors, one of which is, the degree of tissue destruction in the infected area. While, therefore, we may improve upon the methods of serum prophylaxis at present in use, we cannot hope to eliminate the disease completely by the prophylactic use of antitoxin.

Another most important point is suggested by the experiment, viz. that a factor of special import in the prophylaxis of the disease, is early and free removal of devitalised tissue. *Surgery is as important as serum prophylaxis in the prevention of tetanus.*

This finding prompted a further query: "Will a large dose of antitoxin give more adequate protection than a smaller one against a fatal result from infection with *B. tetani* under standard conditions—so far as any infection can be standardised?"

This subject is of great importance and can only be investigated by experiments based on *infection*, as opposed to experiments based on *intoxication*. The point at issue is not how long the passive immunity conferred by a dose of antitoxin lasts, but the degree of immunity conferred by antitoxin during the period between the 3rd and 6th day after the reception of the injury.

It has been my experience in conducting infection experiments in guinea-pigs, that if the animal lives for six days after it has been inoculated without showing evidence of tetanus, the disease will probably not develop at all; or, if it does, the infection, in a considerable number of instances, will only result in local tetanus and subsequent recovery. Stated in terms of the natural disease in man, the question really becomes—*Can we hope to prevent the occurrence of a percentage of fulminating cases of the disease by the routine employment of a larger initial prophylactic dose of antitoxin than that which is at present in use?*

In the earlier series of infection experiments in which guinea-pigs were used, the serum was injected two or three days prior to the inoculation of the infecting material—mixture of spores and irritant—and the following results were obtained:

Table XVII.

No. of animals	Ratio of anti-toxin for use	Units	Recovered	Died
21	1,450	6 or less	0	21
2	2,900	12 units	0	2
6	6,000	25 „	1	5
6	12,000	50 „	2	4

In order further to investigate this problem I performed the following experiment:

Two sets of 3 guinea-pigs each, were immunised with various doses of antitoxin; Lister Institute, No. 136 A serum was used. Two hours before the serum was injected, all the animals were inoculated intramuscularly with a mixture of 0·2 c.c. 1/200 saponin solution + 200 million spores, the total volume of the *inoculum* being 0·6 c.c. Table XVIII, p. 153, shows the details and results of the experiment.

Commenting on these results it is seen, that with the five units of antitoxin the onset of the disease was not delayed so markedly as when larger quantities were employed. With 10 units of antitoxin the late onset of the disease and its relatively slow progress indicate that a larger initial prophylactic dose of antitoxin will probably be of considerable value, both in limiting the incidence of the disease and in beneficially altering its clinical characters, by rendering the latter more chronic and so allowing of more adequate therapeutics.

Table XVIII.

Animal	Saponin	Spores		Anti-toxin	Ratio	Result
A	1/200 0·2 c.c.	200 million Type I		5	1200	Local tetanus 2nd day, generalised 4th day
B	,,	,,	,, I	10	2400	Remained well till 13th day, developed local tetanus—generalised three days later
C	,,	,,	,, I	20	4800	do. do.
A′	,,	,,	,, II	5	1200	Local tetanus 3rd day, definitely generalised 6th day
B′	,,	,,	,, II	10	2400	Remained well till 13th day, developed local tetanus—generalised three days later
C′	,,	,,	,, II	20	4800	Died 4th day. No evidence of tetanus

The experiments are too few in number, and the conditions under which they were carried out too variable—I refer, particularly, to the experiments synopsised in Table XVII, p. 152—to permit of any far-reaching conclusions being drawn from the results obtained; they are, nevertheless, extremely suggestive.

I would here call attention to an apparent discrepancy between the protective values of the sera as shown in the results indicated in Table XVII, p. 152 and Table XVIII, p. 153.

In the experiments shown in Table XVIII the serum appears to exert more marked protective influence than in the experiment shown in Table XVII. This may depend upon the fact, that the serum and the infecting material were inoculated on different days in the first instance, and on the same day in the second instance.

The obvious criticism which might be made of these experiments is, that the ratios of antitoxin used are so high, that the figures are of no import. It must be clearly appreciated, however, that the conditions which determine infection with *B. tetani* in the guinea-pig, are almost certainly very different from those which determine infection in man. One of the most striking features of the infection experiments which I have performed, is the marked degree of tissue debility that must be induced to ensure infection in these animals. It is well known that in unprotected men, on the contrary, a comparatively small lesion may suffice to set up a fatal infection with *B. tetani*.

Further, it is very questionable what interpretations we should put upon the ratios expressed in these Tables, for clearly, a dose of 500 units, for example, will only be neutralised by ten times the quantity of toxin required to neutralise a dose of 50 units. But man is certainly more susceptible to tetanus toxin (spasmin)—probably *much* more susceptible—than the guinea-pig; and there is some ground for believing that he is also much more susceptible to infection. Therefore the ratios stated in Tables XVII and XVIII may not be so fantastic as they appear to be.

Moreover, the extent of the exposed surfaces in large wounds in men, probably tends to make a gramme weight basis of comparison between man and guinea-pig a more correct index of the relative conditions obtaining in the two species, than would a simple "dose for dose" comparison.

It is these factors which make protection experiments in guinea-pigs, when tested by the infection method, extremely difficult to interpret. For with the excessive tissue destruction which is required to ensure infection in that animal, the tetanus bacilli develop in a situation essentially *outside* the body. By reason of their development in this situation, they are protected from the body-fluids, and probably grow with extreme rapidity. In a certain number of cases of the natural disease as it occurs in man, when the tissue lesion is less extensive, the contact between the living tissue and the organism may be fairly intimate. In such circumstances reaction to infection may play a not unimportant part in preventing the occurrence of the disease.

Another point which must not be lost sight of, is, that the concentration of spores in the circumscribed, though relatively large, devitalised area, in experimental infection, is much greater than is likely to occur in most cases of natural infection. This will naturally tend to make the experimental disease exhibit a fulminating character; but unfortunately, a large number of spores—200 million were used throughout the series of tests—appears to be necessary to ensure infection. With small numbers one cannot be certain of introducing a sufficiency of viable spores, to cause death of control unprotected animals within three days from inoculation.

The setting of a time limit of three days for the controls is, of course, arbitrary; but, unless a short period be taken for this purpose, the experimental error assumes serious proportions, owing to the number of survivals which occur, even in the control animals, when the period between inoculation and onset of the disease is lengthened.

Conclusions.

1. As one would naturally expect, the extent and degree of tissue devitalisation are an extremely important factor in determining the occurrence and termination of infection with *B. tetani*.

2. If the extent and degree of devitalisation exceed certain limits, it is certain that in animals—therefore also probably in man—no amount of antitoxin within practical limits, will give complete protection from the disease.

3. There is some evidence that the administration of a large initial prophylactic dose of antitoxin may give adequate protection against tetanus to a larger number of men than a small dose. What I mean is, that while a prophylactic dose of, for example, 500 units, will prevent the occurrence of a certain high percentage of tetanus cases among men, an increased dose to 1000 units would not result in the prevention of double the number of cases.

It would, however, probably increase the percentage of successful applications of serum prophylaxis, although the increase in percentage may be only small.

(*f*) INVESTIGATION OF ANTI-INFECTIVE PROPERTIES OF ANTITOXIC AND ANTI-BACTERIAL SERA.

Experiments undertaken to determine

(i) *Whether antitoxic sera exhibit anti-infective qualities in relation to the serological Type of the infecting bacilli.*

(ii) *Whether improvement in serum prophylaxis may be looked for from the employment of sera possessing anti-bacterial as well as anti-toxic properties.*

The experiments carried out *in vitro* which were discussed in the previous Section of the Report, pp. 120–135, show that *antitoxic* sera neither exhibit specific neutralising properties to the spasm-producing toxins of the three Types, nor do they stimulate phagocytosis of *B. tetani*. The experiments did suggest however, that antitoxic serum might, on the contrary, specifically neutralise the leucotoxic quality possessed by certain specimens of toxin.

It seemed possible, though improbable, that *mono-typical antitoxic* sera might, under certain circumstances, exhibit *anti-infective* properties specific to the Type of the infecting organism.

Experiments were undertaken with a view to investigating this point.

A. INFECTION EXPERIMENTS IN WHICH *WELCHII* TOXIN WAS USED AS THE TISSUE DEBILITANT.

Experiment VIII.

Six guinea-pigs, 1, 2, 3, 4, 5 and 6, were used; animals 1, 2 and 3 each received 2 c.c. normal rabbit serum while 4, 5 and 6 were passively immunised with 2 c.c.—50 units—of the serum of a rabbit prepared with Type II toxin.

On the second day after the administration of the serum, 200 millions of spores were injected by the intramuscular route, together with *Welchii* toxin. The dose of *Welchii* toxin in each instance was 0·1 c.c. = 1/2 the mouse M.L.D. of the particular toxin employed. The details of the experiment and the results obtained are shown in the following Table.

Animal No.	Spores 200 million, *Welchii* toxin 0·1 c.c., volume = 0·6 c.c.	Serum used, volume 2 c.c., antitoxin 50 units	Result
1	Type I	Normal rabbit	Died from tetanus 2nd day after inoculation
2	,, II	,,	do. do.
3	,, III	,,	do. do.
4	,, I	Type II antitoxin	Generalised tetanus 3rd day
5	,, II	,, ,,	Remained well
6	,, III	,, ,,	Generalised tetanus 3rd day

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Experiment IX.

The result of Experiment VIII was encouraging, and it was therefore decided to repeat the test with mice. In this instance, the *antitoxic* sera were mixed with the spores and the *Welchii* toxin, and all three reagents were injected together. The following are the details of the experiment.

Inocula (*a*) spores = 100 million.
„ (*b*) antitoxin = 0·6 units.
„ (*c*) *Welchii* toxin = 1/2 mouse M.L.D.
Antitoxin Type I = Horse 13 B.W.
„ Type II = Rabbit I′.
Inoculation of spores, antitoxin, and tissue debilitant, made together.

Animal No.	Spores Type	Antitoxin Type	*Welchii* toxin	Result
1	I	Nil—saline only	1/2 M.L.D.	Dead 2nd day
2	II	„ „	„	„
3	III	„ „	„	„
4	I	Type I—0·6 units	„	Remained well
5	II	„ „	„	Generalised tetanus 4th day
6	III	„ „	„	„ „ 5th „
7	I	Type II—0·6 units	„	General tetanus 2nd day
8	II	„ „	„	Remained well
9	III	„ „	„	Generalised tetanus 3rd day
10	Nil	Nil	„	Ill for one day but recovered

Note. In the above experiment, the Type I antitoxin was obtained from Dr O'Brien and the vehicle of the antitoxin was therefore horse serum. It is not strictly comparable therefore with the Type II antitoxin which was prepared by myself—the vehicle of the antitoxin being rabbit serum. I here wish to record my thanks to Dr O'Brien for placing this serum at my disposal, as my Type I rabbits were not available for use at that time.

The above results are very striking, but the experiments might justifiably be criticised on the following grounds:

1. It may be purely a coincidence that the *homologous* animals survived. Admittedly the chance of such occurring is remote; but, in view of the complexity of the experiments, it cannot be excluded.

2. Using the toxin of *B. Welchii*—a biological reagent—as the tissue debilitant, introduces a potential error, for it is probable that not every individual of a species is equally susceptible to this toxin. Many attempts have been made to repeat the experiment, but using chemical agents to light up the infection. None of these, however, proved successful; for, either the controls did not take the disease, or the amount of tissue debilitant that had to be introduced to ensure infection, resulted in the death of a number of the animals, not from tetanus, but from poisoning with the chemical agent employed.

3. The amount of *Welchii* toxin used—1/2 M.L.D.—appears to be excessive; but again, it is necessary if infection is to occur regularly. Such a large dose of tissue debilitant leaves but a slight margin to allow of accidents. In an attempt to overcome certain of these criticisms the experiment was repeated on guinea-pigs.

This test was performed one week after the completion of Experiments VIII and IX; the same specimen of *Welchii* toxin was employed, in the same dose, *by volume*, as before. When this experiment was carried out, I did not fully appreciate the rapidity with which *Welchii* toxin deteriorates. As the results of Experiment X show, this deterioration of toxicity really made the test invalid.

I quote the experiment to illustrate one of the many difficulties which are encountered in conducting infection experiments with *B. tetani*, and to emphasise how very difficult it may be to interpret the results obtained.

Experiment X.

The details of this experiment are shown as follows.

(*a*) Animals = guinea-pigs.
(*b*) Spores dose = 250 million.
(*c*) *Welchii* toxin = same dose, and same volume of toxin as used in Experiment VIII.
(*d*) Antitoxin Type I = Horse 13 B.W.
(*e*) „ „ II = Rabbit I'.
(*f*) Dose of antitoxin = 5 units.
(*g*) Method of inoculation = Mixtures made of spores, *Welchii* toxin and tetanus antitoxin, incubated for 30 minutes at 37° C. and injected simultaneously by the intramuscular route.

Animal No.	Spores Type	*Welchii* toxin	Antitoxin 5 units	Result
1	I	„	Nil—saline only	Local tetanus 9th day, generalised 14th day
2	II	„	„ „	General tetanus 8th day
3	III	„	„ „	do. do.
4	I	„	Type I antitoxin	General tetanus 10th day
5	II	„	„ „	Animal remained well
6	III	„	„ „	General tetanus 10th day
7	I	„	Type II antitoxin	General tetanus 10th day
8	II	„	„ „	Animal remained well
9	III	„	„ „	General tetanus 9th day

This experiment is really valueless, for the time which elapsed between making the inoculation and the onset of the disease in the control animals Nos. 1, 2, and 3, was so long, that the passive immunity in the experimental animals must have almost disappeared.

I would call attention to the fact, that both animals 5 and 8 remained well. This suggested that Experiment VIII should be accepted with a certain reserve, as it appeared possible, that the infectivity of the Type II spores used might be somewhat less than that of the Type I and Type III spores. In view of the result of Experiment IX, I do not think that this was the

case in Experiment VIII, but the possibility of such an interpretation of the results at any stage of the investigation, must be borne in mind.

The experiment was then repeated, and on this occasion sufficient *Welchii* toxin was administered to cause swelling of the whole of the inoculated limb. The result was that none of the animals survived, and the protected animals took the disease almost as soon as the control animals. These tests on guinea-pigs and three other tests which were carried out on mice, convinced me, that one could not hope to obtain consistent results if *Welchii* toxin were employed as the tissue debilitating factor in the mixtures, owing to the difficulty of regulating the dose.

Note. Experiments VIII, IX, and X, and their repetitions were carried out with sample bleedings—two different samples were used—from Rabbit I′, while the animal was still undergoing immunisation. Before the next series of tests was made the animal had been bled out.

It was at this stage of the investigation that I decided to change my technique, and in guinea-pigs at least, to employ chemical reagents as the tissue debilitant factor of the infecting mixtures.

Several chemical agents were tried, but saponin gave the most constant results, and was fairly easily handled, so that this substance was chosen for future investigations.

B. Experiments in which Saponin was used as the tissue debilitant and the anti-infective quality of antitoxin was under investigation.

Experiment XI.

In this experiment the animals were passively immunised with 25 units of antitoxin, and two days later, were inoculated by the intramuscular route with a mixture of saponin and spores.

(*a*) Animals = guinea-pigs.
(*b*) Type I antitoxin = B.W. Horse 13.
(*c*) „ II „ = Rabbit I′.
(*d*) Antitoxin dose = 25 units.
(*e*) „ administered 48 hours before inoculations were made.
(*f*) Spores = 200 million.
(*g*) Saponin = 0·2 c.c. of 1/200 dilution.
(*h*) Volume of infecting *inoculum* = 0·6 c.c.

Animal No.	Spores Type	Saponin	Antitoxin units 25	Result
1	I	0·2 c.c. 1/200	Nil—saline only	Generalised tetanus 2nd day
2	II	„	„ „	„ „ „
3	III	„	„ „	„ „ „
4	I	„	Type I	Generalised tetanus 11th day
5	II	„	„	Generalised tetanus 3rd day
6	III	„	„	„ „ „
7	I	„	Type II	„ „ „
8	II	„	„	„ „ „
9	III	„	„	„ „ „

The experiment was then performed by the method of making simultaneous inoculation of serum, debilitant and spores. The details are as follows.

Experiment XII.

(*a*) Animals = guinea-pigs.
(*b*) Spores = 200 million.
(*c*) Saponin = 0·1 c.c. of 1/100.
(*d*) Antitoxin = 5 units.
(*e*) Total volume of *inoculum* = 0·6 c.c.
(*f*) Inoculation of antitoxin and infecting mixture made simultaneously.
(*g*) Antitoxic sera employed were the same as in Experiment XI.

Animal No.	Spores Type	Saponin	Antitoxin 5 units	Result
1	I	0·1 c.c. of 1/100	Nil—saline only	Generalised tetanus 2nd day
2	II	,,	,, ,,	,, ,, ,,
3	III	,,	,, ,,	,, ,, ,,
4	I	,,	Type I	Animal developed local tetanus 14th, generalised 15th day
5	II	,,	,,	Generalised tetanus 3rd day
6	III	,,	,,	,, ,, ,,
7	I	,,	Type II	Generalised tetanus 2nd day
8	II	,,	,,	,, ,, 4th ,,
9	III	,,	,,	,, ,, 2nd ,,

That portion of the experiment dealing with Type II antitoxin was repeated twice as the above result did not corroborate Experiments VIII and IX in which *Welchii* toxin had been used as the tissue debilitant. Both experiments failed to show definite protection against infection; either *general*, in relation to all the Types, or specific, in respect of the *homologous* Type.

Examination of this serum showed that it was contaminated with a diplococcus. The serum was filtered, and the experiments were discontinued until such time as complete crossed experiments could be performed, using both *anti-bacterial* and *antitoxic* sera.

In these experiments the following points call for comment:

1. I failed to corroborate the findings of Experiment VIII in respect of Type II serum, both when the serum was administered before inoculation of the infecting mixture and when it was administered simultaneously therewith.

2. It is significant, on the other hand, that Type I antitoxin did protect for a much longer period against infection with Type I spores, than did Type II antitoxin.

3. It is remarkable that a dose of 5 units of antitoxin, when administered simultaneously with the infecting mixture, appears to give almost as much protection against infection, as does a dose of 25 units of the same antitoxin administered two days prior to the inoculation of the infecting mixture.

Before leaving this subject I decided to repeat Experiment VIII, using Type I antitoxin, and saponin as the irritant. My reason for so doing was, that in neither Experiment XI nor XII did I succeed in obtaining an unequivocal result. In performing this further experiment, the test animals

were passively immunised with 50 units of antitoxin and were inoculated with the saponin-spore mixture two days later.

Experiment XIII.

(*a*) Animals = guinea-pigs.
(*b*) Spores = 200 million.
(*c*) Saponin = 0·1 c.c. of 1/100 dilution.
(*d*) Volume of *inoculum* = 0·6 c.c.
(*e*) Antitoxin used = B.W. Horse 13.
(*f*) Dose of antitoxin = 50 units.
(*g*) Antitoxin administered two days before infecting inoculations were made.

Animal No.	Spores Type	Saponin	Antitoxin 50 units	Result
1	I	0·1 c.c. of 1/100	Nil—saline only	General tetanus 2nd day
2	II	,,	,, ,,	,, ,, ,,
3	III	,,	,, ,,	,, ,, ,,
4	I	,,	Type I B.W. 13	Remained well till 12th day, died 13th, streptococci recovered from local lesions and internal organs
5	II	,,	,,	Local tetanus 4th day, generalised 8th day
6	III	,,	,,	Local tetanus 4th day, generalised 5th day

The following deductions seem permissible from experiments VIII–XIII, pp. 155–160:

1. That *antitoxic* serum may under certain conditions exhibit *anti-infective* properties; and

2. While the experiments afford no *proof* that a monotypical antitoxic serum protects more adequately against *infection* due to serologically *homologous* strains of the bacillus than against that due to *heterologous* strains, the results obtained nevertheless *suggest* that protection against the *homologue* is more adequate.

These deductions are made in a spirit of modesty, for one is well aware that, in infection experiments, and especially with the anaërobes, even the known factors upon which the process depends are very complex. Short of obtaining a 100 per cent. result in a considerable series of experiments, it is rash to conclude that proof of any thesis has been established.

Does serum possessing anti-bacterial properties exhibit more adequate prophylactic qualities than serum which possesses only antitoxic (anti-spasmin) properties?

Note. While the term "unit" as previously employed refers to the U.S.A. Unit, I wish especially to call attention to the fact that, in the experiments of the *following Sub-section*, the term "unit" is defined as "that quantity of antitoxin which will protect a mouse against 1000 M.L.D. of tetanus toxin."

The investigation of this problem involved an inquiry into the following points:

(i) Does serum possessing demonstrable *anti-bacterial* qualities (*e.g.* agglutinin and phagocyte stimulating properties) but of no, or only negligible antitoxic value, exhibit any capacity for preventing *infection*?

(ii) We know that *antitoxin* has a certain *anti-infective* power. Can this be enhanced by adding serum containing *anti-bacterial* immune bodies? If antitoxic (anti-spasmin) content be made the basis of assessing the probable prophylactic value of sera, does a serum of x "anti-spasmin" units which has no *anti-bacterial* qualities, protect against infection as effectively as a serum of x "anti-spasmin" units, which does possess *anti-bacterial* properties?

(iii) Can specific protection be demonstrated *in vivo* with such *anti-bacterial* sera?

The consideration of these points is of extreme import; for, should any of these questions be answered in the affirmative, the method of standardising prophylactic sera will demand review. It is only rational that, if a satisfactory method could be devised for examining sera designed for prophylactic use, these sera should be standardised on the basis of prevention of *infection*, rather than of *intoxication*.

The first experiments made were designed to corroborate the findings already obtained, and to determine a method of procedure which could be satisfactorily used for the purpose in view.

Experiment XIV.

This experiment was carried out as a preliminary to more careful and exact tests, in which guinea-pigs were to be used. In this instance mice were the experimental animals employed. The tissue irritant chosen was therefore *Welchii* toxin and a complete series of crossed tests was made, both with monotypical *antitoxic* and monotypical *anti-bacterial* sera corresponding to three serological Types of *B. tetani*.

The details of the experiments are as follows:

(*a*) Animals = mice.
(*b*) Tissue irritant = *Welchii* toxin $< 1/2$ but $> 1/4$ M.L.D.
(*c*) Spores = 100 million.
(*d*) Inoculation = *Sub cutem.*
(*e*) *Antitoxic* sera dose = 1 "unit."
(*f*) *Anti-bacterial* sera dose = antitoxic content of < 1 "unit" and $> 1/2$ "unit."
(*g*) Sera administered one day prior to inoculation of the infecting mixtures.
(*h*) Volume of infecting *inoculum* = 0·2 c.c.

Type I Spores.

1.	*Welchii* toxin nil	Serum nil	Died 5th day, no evidence of tetanus
2.	*Welchii* toxin	+serum nil	Remained well
3.	,, ,,	+Type I *antitoxin*	,, ,,
4.	,, ,,	+ ,, II ,,	Died 2nd day—? tetanus
5.	,, ,,	+ ,, III ,,	,, ,, ,,
6.	,, ,,	+ ,, I *anti-bacterial*	Remained well
7.	,, ,,	+ ,, II ,,	,, ,,
8.	,, ,,	+ ,, III ,,	Died 2nd day—? tetanus

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Type II Spores.

1.	*Welchii* toxin nil	Serum nil	Remained well
2.	*Welchii* toxin	„	Definite tetanus 3rd day, died
3.	„ „	+ Type I *antitoxic* serum	Definite tetanus 4th day
4.	„ „	+ „ II „ „	Remained well
5.	„ „	+ „ III „ „	„ „
6.	„ „	+ „ I *anti-bacterial* serum	„ „
7.	„ „	+ „ II „ „	„ „
8.	„ „	+ „ III „ „	Definite tetanus 3rd day

Type III Spores.

1.	Nil	Nil	Died 4th day, no evidence of tetanus
2.	*Welchii* toxin	„	Definite tetanus 3rd day
3.	„ „	+ Type I *antitoxic* serum	„ „
4.	„ „	+ „ II „ „	„ „
5.	„ „	+ „ III „ „	Remained well
6.	„ „	+ „ I *anti-bacterial* serum	Died 3rd day, ? evidence of tetanus
7.	„ „	+ „ II „ „	Remained well
8.	„ „	+ „ III „ „	„ „

The results of Experiment XIV may be summarised thus:

(i) Of those animals which received spores *homologous* to antitoxic sera employed, all three remained well.

(ii) Of six which received spores *heterologous* to the antitoxin three developed definite tetanus and died; two others died but evidence of death from tetanus was lacking, and one remained well.

(iii) Of nine animals which received *anti-bacterial* sera, one showed definite tetanus; two died but evidence of tetanus was lacking, and six remained well.

Considering that the unitage of the *anti-bacterial* sera in the above experiment was certainly less than that of the *antitoxic* sera, it is notable that so many survivals occurred among the animals protected with the former.

The section of the experiment which deals with Type I spores is, however, really valueless, in that infection did not "take" in the control.

If then we consider only those sections of this experiment which deal with Type II and Type III spores, it is seen that

(*a*) Of six mice protected with *antitoxic* sera, three developed tetanus and three remained well.

Two of the surviving mice were protected by sera *homologous* to the infecting organisms; in one the infection was *heterologous*.

(*b*) Of six animals protected by *anti-bacterial* sera, only one developed tetanus and four remained well. One mouse died on the third day after inoculation but evidence of death from tetanus was lacking. Both of those in which the sera and the infecting organisms were *homologous* were included among the four mice which remained well.

Here again is a suggestion, but no proof, that *monotypical* antitoxic sera may, under certain circumstances, exhibit a specific influence on infection from *serologically homologous* bacilli.

(*c*) There is also an indication that *anti-bacterial* sera exhibit more adequate *anti-infective* properties than do *antitoxic* sera, provided that the "anti-spasmin" value of the sera be made the basis of comparison.

Note. There is some ground for believing that mice are not very susceptible to tetanus *infection*. When these animals are employed, the assessment of the results obtained is rendered especially difficult.

Far-reaching conclusions cannot be drawn from the results obtained.

The above experiment, which lays no claim to strict accuracy, was valuable however, in that it served to indicate how to proceed further and showed that the thesis advanced was worthy of extended investigation.

Henceforth guinea-pigs only were employed as the experimental animals, and the irritant used was saponin. More reliable results could be expected with this technique, than when mice and *Welchii* toxin were used.

Experiment XV.

This experiment was carried out to determine whether *anti-bacterial* sera, possessing little or no antitoxic properties, would protect against *infection*. Sample bleedings were made from Rabbits III, III′ and III″, "anti-whole culture" serum to Types I, II and III respectively. These sera, retained from a previous investigation, showed some phagocytic properties when diluted 1/200, and their antitoxic value was equal to about 2 "units" per c.c. With these serum samples the following experiment was carried out.

(*a*) Animals = guinea-pigs.
(*b*) Spores = 200 million.
(*c*) Tissue irritant = 0·1 c.c. of 1/100 saponin.
(*d*) Inoculation = intramuscular.
(*e*) *Anti-bacterial* sera Type I, Type II and Type III (equivalent of 1 "anti-spasmin" "unit" used).
(*f*) *Antitoxic* serum = Type I (equivalent of 6 "anti-spasmin" "units" used).
(*g*) Sera administered one day prior to inoculation of infecting mixture.
(*h*) Volume of infecting *inoculum* = 0·6 c.c.

Animal No.	Spores	Serum	Result
1	I	Nil	General tetanus 4th day
2	II	,,	,, ,, 3rd ,,
3	III	,,	Animal remained well
4	I	6 "units" Type I *antitoxic*	General tetanus 7th day
5	II	,, ,, ,,	,, ,, 3rd ,,
6	III	,, ,, ,,	,, ,, 7th ,,
7	I	1 "unit" Type I *anti-bacterial*	,, ,, 3rd ,,
8	II	,, ,, ,,	,, ,, 3rd ,,
9	III	,, ,, ,,	,, ,, 3rd ,,
10	I	1 "unit" Type II *anti-bacterial*	,, ,, 4th ,,
11	II	,, ,, ,,	,, ,, 2nd ,,
12	III	,, ,, ,,	,, ,, 3rd ,,
13	I	1 "unit" Type III *anti-bacterial*	,, ,, 3rd ,,
14	II	,, ,, ,,	,, ,, 2nd ,,
15	III	,, ,, ,,	,, ,, 3rd ,,

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Experiment XV shows that sera which possess *anti-bacterial* properties will not serve to prevent a fatal issue in an experimental infection set up by the method described, if the sera do not also possess sufficient *antitoxic* qualities.

This fact was confirmed by investigations made with agglutinating sera which possessed no, or negligible *antitoxic* qualities. These also failed to prevent infection under the conditions of the experiment.

I wish to call attention to the survival of the control animal which received Type III spores. In view of such survivals, extreme care must be exercised in drawing conclusions from the results of these tests.

Experiment XV was repeated with a modified technique; serum and infective material being injected simultaneously, and only *anti-bacterial* sera used. The quantity of each serum used contained 1 "unit" of antitoxin.

The result of this experiment showed, that even under these conditions, the *anti-bacterial* sera did not exert demonstrable *anti-infective* qualities in the experimental *infection* set up by the method described.

This result was somewhat unexpected; but, when it is borne in mind that saponin has an extremely deleterious influence on all the tissues, and is very markedly haemotoxic, this can be readily understood. For, during the first day or two after inoculation, *B. tetani* develops virtually outside the body, and may elaborate enough toxin to kill the animal before sufficient local reaction is set up in the tissues to deal with the process of infection. If it were possible to combat this initial intoxication, and at the same time favourably influence the tissues to deal with the local infective process, better results might be obtained.

With this object in view the following experiment was undertaken.

Experiment XVI.

Four guinea-pigs, A, B, C and D, each received 20 "units" of Type III antitoxin (*i.e.* antitoxin prepared by inoculation of the toxin of Type III bacilli).

The following day

A was inoculated with a mixture of saponin and Type I spores.
B ,, ,, ,, ,, I ,,
C ,, ,, ,, ,, III ,,
D ,, ,, ,, ,, III ,,

Two days were allowed to elapse and then,

A was given a dose of 2 "units" of Type III *anti-bacterial* serum.
B ,, ,, 2 ,, ,, *antitoxic* ,,
C ,, ,, 2 ,, ,, *anti-bacterial* ,,
D ,, ,, 2 ,, ,, *antitoxic* ,,

Animals A, C and D remained well, while B developed generalised tetanus

six days after the inoculation of the infective mixture. In this experiment then,

(*a*) The animal which received *homologous* antitoxin survived.

(*b*) The animal which received only *heterologous* antitoxin died from tetanus.

(*c*) Both animals, which on the second day after inoculation of infective material received a dose of *anti-bacterial* serum, recovered.

In this experiment too large an initial dose of antitoxin was used to permit of its being satisfactorily demonstrated that *anti-bacterial* sera possess more adequate *anti-infective* properties than do *antitoxic* sera.

In Experiment XVII an effort was made to overcome this difficulty by using a smaller dose of serum.

Experiment XVII.

Four guinea-pigs A, B, C and D were passively immunised as follows:

A received 12 "units" of Type III *antitoxic* serum
B „ 6 „ „ „ +4 "units" of Type III *anti-bacterial* serum
C „ 12 „ „ *antitoxic* serum
D „ 6 „ „ „ + 4 "units" of *anti-bacterial* serum

The following day A and B were each inoculated intramuscularly with a mixture of saponin 0·1 c.c. of 1/100 and Type I spores 200 million, total volume 0·6 c.c. C and D each received a similar inoculation, but with Type III instead of Type I spores.

Animal A developed local tetanus on the third day after inoculation, the disease being generalised on the fifth day.
„ B developed local tetanus on the fifth day which generalised on the sixth day.
„ C developed local tetanus on the third day, general tetanus supervening on the following day.
„ D remained well.

This experiment definitely showed that *anti-bacterial* sera were worthy of extended investigation.

I would call attention to the fact, that Experiments XVI and XVII illustrate a further difficulty, which has so far not been overcome, viz. that the bacilli, from a given number of spores, are not always equally toxogenic. In Experiment XVI, Animal B (inoculated with Type I spores), although it received in all 22 "units" of antitoxin, developed generalised tetanus six days after inoculation. Whereas in Experiment XVII when the same spores, but only half the quantity of the same antitoxin, were used, generalised tetanus developed only one day earlier—the fifth day—preceded by local tetanus for a period of two days. In Experiment XVII then, the Type I spores did not appear to be so toxogenic as in Experiment XVI.

In order, finally, to examine this subject, a complete series of experiments was carried out. Great care was taken to standardise the reagents as accurately as the equipment of my laboratory would permit, and to carry out the experiments under standard conditions as far as possible.

The following are the protocols of experiments carried out with the specimen

bleedings of *antitoxic* and *anti-bacterial* sera. The sera were standardised with fair accuracy before the tests were performed.

As it was not possible to carry out the entire experiment with all six sera —three *antitoxic* and three *anti-bacterial*—on the same day, the experiment had to be divided into three sections. Each section deals with the investigation of the *anti-infective* value of all the sera in respect of only one Type of spores.

METHODS USED.

(1) The basis of comparison for the sera was their *antitoxic* value. This was standardised as accurately as possible; but no doubt slight differences existed between the real and the estimated unitage of each. I was unable to obtain any standard U.S.A. toxin with which to perform the preliminary tests and therefore had to adhere to the "mouse M.L.D." method of standardisation. Such differences, however, do not really affect the results, as the same ratio obtains in each experiment of the series.

(2) The quantity of antitoxin provisionally decided upon was 10 units.

(3) The sera were injected *sub-cutem* into the right hind limb.

(4) One hour after the administration of the sera, a mixture consisting of 0·1 c.c. 1/100 dilution of saponin + 200 million spores suspended in 0·6 c.c. of saline, was injected intramuscularly into the left hind limb.

(5) The animals employed were guinea-pigs.

(6) The volume of serum administered in each instance was made up to 2 c.c. by the addition of normal rabbit serum. (The *antitoxic* and *anti-bacterial* sera used in these experiments were obtained from later bleedings from the same rabbits as were used in the experiments described in Section IV.)

(7) The site of the inoculation where the saponin spore mixture was injected was dried with alcohol and sealed with collodion. This precaution is essential, because of the danger of secondary infection from the animal cage, bedding, etc. This danger is enhanced by the tissue devitalisation produced by the saponin.

Experiment XVIII.

(*a*) Type I spores used.

(*b*) Antitoxic titre of each serum tested—10 "units."

Number	Serum	Result
1	Normal rabbit serum 2 c.c.	Died from tetanus 3 days after inoculation
2	Type I *antitoxic* serum 10 "units"	Local tetanus developed 3rd day; persisted for 8 days. Thereafter the animal slowly recovered
3	Type II *antitoxic* serum 10 "units"	Local tetanus developed 3rd day; persisted for over three weeks
4	Type III *antitoxic* serum 10 "units"	Local tetanus 3rd day; generalised on 4th; animal killed
5	Type I *anti-bacterial* serum 10 "units"	Animal remained well
6	Type II *anti-bacterial* serum 10 "units"	Local tetanus 3rd day; generalised 4th; animal killed
7	Type III *anti-bacterial* serum 10 "units"	Local tetanus 4th day; generalised 5th; animal killed

Experiment XIX.

(*a*) Type II spores used.

(*b*) Antitoxic content of each serum—7·5 "units." (In view of the survival of animals 2 and 3 in Experiment XVIII the smaller dose—7·5 "units of" antitoxin—was decided upon.)

Number	Serum	Result
1	Normal rabbit serum 2 c.c.	Died from tetanus 3 days after inoculation
2	Type I *antitoxin* 7·5 "units"	Local tetanus 3rd day; generalised 4th; animal killed
3	Type II *antitoxin* 7·5 "units"	Local tetanus 3rd day; generalised 4th; animal killed
4	Type III *antitoxin* 7·5 "units"	Local tetanus 3rd day; both hind legs tetanised from 5th–12th day; animal commenced to use both hind limbs 17th day; recovered
5	Type I *anti-bacterial* serum 7·5 "units"	Local tetanus 3rd day; generalised 4th; animal killed
6	Type II *anti-bacterial* serum 7·5 "units"	Remained well
7	Type III *anti-bacterial* serum 7·5 "units"	Local tetanus 3rd day; generalised 4th; animal killed

Experiment XX.

(*a*) Type III spores used.

(*b*) Antitoxic content of each serum—10 "units."

[The higher dose of antitoxin was reverted to in this instance, as previous experiments with Type III spores had indicated that these were probably markedly toxogenic.]

Number	Serum	Result
1	Normal rabbit serum 2 c.c.	Local tetanus 2nd day; generalised tetanus 3rd day; killed
2	Type I *antitoxin* 10 "units"	Local tetanus 2nd day; generalised tetanus 3rd day; killed
3	Type II *antitoxin* 10 "units"	Local tetanus 3rd day; did not generalise; remained for 10 days; thereafter animal slowly recovered
4	Type III *antitoxin* 10 "units"	Local tetanus 3rd day; did not generalise; remained for 10 days; thereafter animal slowly recovered
5	Type I *anti-bacterial* serum 10 "units"	Local tetanus 3rd day; did not generalise; remained for 8 days; thereafter slowly recovered
6	Type II *anti-bacterial* serum 10 "units"	Local tetanus 3rd day; generalised 4th day; animal killed
7	Type III *anti-bacterial* serum 10 "units"	Local tetanus 3rd day; generalised 4th day; animal killed

This result fails to corroborate Experiments XVIII and XIX. Animals 3 and 5, although only immunised with *heterologous* sera, survived for a longer period than the animal which received *homologous anti-bacterial* serum.

It is to be noted that animals 3, 4 and 5 were all very small guinea-pigs; they exhibited the same markings, and appear to have been, though the evidence is not definite, from the same litter. It has been noted throughout the whole series of infection experiments that small guinea-pigs are

less susceptible and give less constant results than larger animals. This probably because it is more difficult to ensure an intramuscular injection being made when the animals are small. Attention is therefore especially called to the fact that these animals which survived in Experiments XVIII and XIX were comparable to those which died in Experiment XX. Owing to lack of animals, I was unable to obtain nine which were really comparable to one another.

A further series of experiments was therefore carried out using larger animals. The animals in each test were of approximately equal weight.

Owing to a shortage of guinea-pigs these tests were confined to an examination of three sera only, each serum being examined in triplicate, so that a range of unitage could be employed in the investigation of each.

The sera examined were:

(*a*) *Antitoxic* serum = Type III.
(*b*) *Anti-bacterial* serum = Type II.
(*c*) *Anti-bacterial* serum = Type III.

Experiment XXI.

The details of this experiment are as follows:

(*a*) Animals = guinea-pigs of approximately 350 grams.
(*b*) Spores = Type III—200 million.
(*c*) *Anti-spasmin* "unitage" in each case, 9, 12 and 15.
(*d*) Irritant = Saponin, 1/100, 0·1 c.c.
(*e*) Volume of *inoculum* = 0·6 c.c.
(*f*) Serum administered one hour before injection of spore-saponin mixture.

In this experiment, the *anti-bacterial* value of the sera employed remained constant in each test, the equivalent of 6 "units." The balance of *antitoxin* was made up of 3, 6 and 9 U.S.A. Units of a polyvalent antitoxic horse serum marked L.I.P.M. 136 A.

The following were the results obtained

Animal No.	Serum administered	Spores	Result
1	6 "units" Type III *antitoxin* (rabbit), 3 units L.I.P.M. 136 A	Type III	General tetanus 2nd day
2	6 "units" Type III *antitoxin* (rabbit), 6 units L.I.P.M. 136 A	,,	,,
3	6 "units" Type III *antitoxin* (rabbit), 9 units L.I.P.M. 136 A	,,	,,
4	6 "units" Type II (rabbit) *anti-bacterial*, 3 units L.I.P.M. 136 A	,,	,,
5	6 "units" Type II (rabbit) *anti-bacterial*, 6 units L.I.P.M. 136 A	,,	,,
6	6 "units" Type II (rabbit) *anti-bacterial*, 9 units L.I.P.M. 136 A	,,	,,
7	6 "units" Type III (rabbit) *anti-bacterial*, 3 units L.I.P.M. 136 A	,,	,,
8	6 "units" Type III (rabbit) *anti-bacterial*, 6 units L.I.P.M. 136 A	,,	,,
9	6 "units" Type III (rabbit) *anti-bacterial*, 9 units L.I.P.M. 136 A	,,	Local tetanus 2nd day General tetanus 4th day

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This experiment will be commented on later; but I wish to call attention to the somewhat longer survival of animal 9 as compared with animals 3 and 6.

Experiment XXII.

In this experiment though similar to the previous one horse serum was not added to bring up the desired *anti-spasmin* content of the *anti-bacterial* sera. Both *anti-spasmin* and *anti-bacterial* content therefore varied *pari passu* one with the other.

The details of the test are substantially the same as those of Experiment XXI but 10, 20 and 30 "units" were employed instead of 9, 12 and 15.

Animal No.	Serum administered	Spores	Result
1	Type III rabbit *antitoxin* 10 "units"	Type III	General tetanus 2nd day
2	Type III rabbit *antitoxin* 20 "units"	,,	,, ,, ,,
3	Type III rabbit *antitoxin* 30 "units"	,,	,, ,, 3rd day
4	Type II rabbit *anti-bacterial* 10 "units"	,,	,, ,, 2nd ,,
5	Type II rabbit *anti-bacterial* 20 "units"	,,	,, ,, ,,
6	Type II rabbit *anti-bacterial* 30 "units"	,,	Local tetanus 3rd day General tetanus 4th day
7	Type III rabbit *anti-bacterial* 10 "units"	,,	Local tetanus 2nd day General tetanus 3rd day
8	Type III rabbit *anti-bacterial* 20 "units"	,,	Local tetanus 2nd day General tetanus 3rd day
9	Type III rabbit *anti-bacterial* 30 "units"	,,	Local tetanus 3rd day General tetanus 4th day

I shall not comment on these results beyond calling attention to the fact, that it is obvious, that if specificity of reaction between serum and organism exists, it is obviously *quantitative*. Experiments XVIII, XIX, XX, XXI and XXII all show the difficulty of realising the conditions necessary for the demonstration of specificity.

SYNOPSIS OF RESULTS OBTAINED IN SECTION V (pp. 135–169).

The results obtained in the Section of the Report at present under consideration may be summarised thus:

1. The genesis of tetanus *infection* induced by experimental methods depends very largely upon the degree and extent of the tissue debility produced by the *inoculum*.

2. The nature of the tissue debilitant employed for setting up infection is an important factor in this connection; for a debilitant which lights up infection in one animal species may fail to do so in another. Thus, when mice are inoculated with mixtures of saponin and tetanus spores, these animals only occasionally develop the disease. On the contrary, when guinea-pigs are inoculated with such mixtures they seldom survive, the onset of the disease being usually early and its character fulminant.

3. The quality of the tissue debility induced is also of paramount impor-

tance in the genesis of infection. Thus trimethylamine, even though it be used in sufficient concentration to cause the development of large eschars at the site of inoculation, when injected together with tetanus spores into mice only infrequently lights up the disease; whereas the toxin of *B. Welchii*, injected in sub-lethal dose together with the same spores, almost invariably sets up tetanus in these animals, notwithstanding the fact that no obvious lesion could be demonstrated *ante-mortem*.

4. In animals protected by *antitoxin*, a rapidly fatal tetanus infection can be induced, provided that the spore-containing *inoculum* produce a sufficient degree of tissue debility of the requisite character.

5. In the case of infections arising from Type I and Type II spores (Type III spores were not included in the test), it was shown that in guinea-pigs, by increasing the prophylactic dose of *antitoxic* serum, the onset of the disease can be delayed and its course can be rendered less acute. If the increase of prophylactic dose be sufficient, *antitoxic* serum *may* completely protect these animals against an *infection* induced by the inoculation of "saponin-spore" mixture.

6. There is some evidence that *monotypical antitoxic* sera protect more adequately against infection with bacilli which are serologically *homologous*, than against infection due to bacilli which are serologically *heterologous*.

Admittedly, the experimental evidence of this is only suggestive, and this deduction is made with great reserve.

7. The experiments on the relative prophylactic value of *antitoxic* and *anti-bacterial* sera prove that this subject is worthy of careful investigation.

8. Experiments XVIII and XIX (pp. 166, 167) in which Type I and Type II spores were used, strongly suggest that sera possessing *anti-bacterial* as well as *antitoxic* properties protect more adequately than do sera exhibiting only *antitoxic* properties. The evidence of specific protection in relation to the serological Type of the infecting organism appears also to be definite in these two experiments.

9. Experiment XX (p. 167), in which Type III spores were used, failed to corroborate this finding. The experiment is however interesting, in that it indicates that there is a considerable variation of susceptibility to tetanus infection among animals of one species.

10. Experiments XXI and XXII (pp. 168, 169) give a very slight indication that in the case of Type III also, *anti-bacterial* serum affords more adequate protection than does *antitoxic* serum. There is also a suggestion in the results obtained that the protection is specific.

DISCUSSION OF THE RESULTS OF EXPERIMENTS OF SECTION V.

It is difficult to comment upon the results obtained in Section V, for, although suggestive, they fall very far short of establishing the validity of the thesis advanced. I wish then formally to call attention to the fact that

any deductions which have been drawn from the results so far obtained, are to be regarded as only provisional in character.

The following criticisms are applicable to the experiments described.

(*a*) The number of experiments performed is too small to permit of definite information being obtained on the points at issue.

(*b*) The method employed for setting up infection is unsatisfactory for the following reasons.

(i) To set up tissue destruction or debility by means of saponin is a highly artificial procedure.

(ii) The disturbance, which must be produced when this reagent is the debilitant employed, is so great (if infection in the control animals is to be assured), that it is extremely difficult to protect against infection, even when relatively enormous doses of serum are used prophylactically.

This large amount of tissue destruction really means that the inoculated spores develop in a situation relatively remote from the body-fluids, and in such circumstances may be protected from immune bodies circulating in the blood or lymph. As the germinated bacilli continue to develop toxin, any antitoxin that is administered may ultimately be exhausted, and tetanus supervenes.

The method which one is forced to employ has therefore grave disadvantages, and leads, probably, to too severe a test being applied to the sera examined.

(iii) It is obvious that a standard method of infection is an unattainable ideal, for spores certainly vary in respect both of their *toxogenic* capacity and of their *infective* capacity.

It is to be noted that the two factors of *toxogenicity* and *infectivity* are not identical, for the spores from some cultures which have a relatively low toxin content may, under certain circumstances, be more highly *infective* than are spores from a culture of greater toxicity.

(iv) When all other sources of error which render comparative experiments of the kind under consideration difficult to carry out are eliminated, there remain two factors which never can be standardised.

(*a*) The test animals will always exhibit idiosyncrasy.

(*b*) The *toxogenic* capacity, and also probably the *infective* capacity, of a single strain of *B. tetani* varies from culture to culture.

A very important question arises in connection with this section of the work, viz. "Can we hope for any improvement in the serotherapeutics of declared tetanus by the use of *anti-bacterial* sera?"

While it is unfortunately probable that this question will have to be answered in the negative, experiments dealing with the problem will have to be undertaken.

These experiments cannot be carried out until larger quantities of *anti-bacterial* sera are available; but arrangements are now being made for the immunisation of horses against *whole culture*. The problem will

therefore be dealt with when sufficient serum from these animals is obtained.

Since completing the experiments described in Section V, I have received information, communicated personally, from Capt. Bullock, R.A.M.C., Imperial Cancer Research Laboratories, concerning the influence of the calcium ion in initiating anaërobic infections.

I here wish to record my very deep debt to Capt. Bullock, R.A.M.C., for the information which he has placed unreservedly at my disposal before the publication of his work.

Preliminary experiments with soluble calcium salts as the "Infection initiator" both in mice and in guinea-pigs have given encouraging results; and, in view of the slight tissue destruction which occurs when calcium salts are used for this purpose, I propose to repeat Experiments XVIII, XIX and XX using Bullock's technique.

SECTION VI.

INVESTIGATION OF DRESSINGS IN RELATION TO ANAËROBIC INFECTIONS OF WOUNDS.

In this section of the Report are considered the results obtained in the examination of 100 wounds during the process of healing.

The object of the inquiry was to determine, if possible, the procedure or procedures, which might be recommended as useful for preventing or diminishing infection of wounds due to anaërobic bacteria.

In view of the influence which anaërobes have one upon another, the general question of all anaërobe infection, rather than the particular question of any one such infection, was investigated. The presence of *B. tetani* was, however, made the subject of detailed inquiry, as the prevalence of the various serological Types of that organism, and its incidence in wounds, constitute a most important aspect of the bacteriology of tetanus.

Owing to its being relatively easy to differentiate morphologically, and because also of its important relationship to the etiology of tetanus, the incidence of *B. Welchii* was also noted. No effort could be made, however, to determine the incidence of other anaërobes in the material examined.

(*a*) *Methods used.*

It was felt, that in making this investigation, it would be better to examine thoroughly a relatively small number of wounds, and to examine them repeatedly, than to make a single examination of a much larger number. The progress made under the various treatments could be more definitely assessed by choosing a small number of cases, that were more or less comparable to one another; that is, in so far as comparison of wounds is at all possible. Five examinations of each wound were made at weekly intervals,

unless the patient became convalescent during the period. The examinations were conducted as follows:

(i) Swabs were taken as soon after the arrival of a convoy as possible.

(ii) Thereafter the same wounds were examined at intervals of approximately seven days, for a period of five weeks.

The material of each swab was inoculated into meat water tubes—"*a*" and "*b*." The tubes were boiled prior to inoculation, in order to ensure anaërobiasis.

Tube "*a*" was incubated anaërobically to obtain a growth of non-sporulating organisms.

Tube "*b*" was heated to 65° C. for 30 minutes before incubation, in order to obtain growths of sporing anaërobes only.

Each culture was examined ten times at intervals of three or four days.

When the anaërobes developed rapidly, and in some variety, the swab was marked in the records as "heavily infected"; and when the cultures showed only a slight or medium growth, the result was noted as "light anaërobe infection."

It might be argued that a notation of this kind is arbitrary, but only by taking a broad view, could one preserve a correct perspective of the results obtained. In Diagrams XIII, XIV, XV, XVI and XVII, pp. 191–195, are given the details of the examination of each swab.

(iii) When organisms, having the morphological characters of *B. tetani*, appeared in the meat cultures, these were sub-cultured into the "exhausted" medium described in previous communications, and the growth therefrom tested by the agglutination methods. When a culture was obtained that agglutinated in the presence of any of the type sera, its toxogenicity was then tested by sub-culture from the original meat tube, and, in certain instances, its infective quality was also examined.

(iv) The tubes "*a*," incubated without previous heating, were examined with a view to demonstrating the presence of *B. Welchii*.

Note. This procedure was only introduced after swabs from 36 cases had already been examined. The figures relating to *B. Welchii* deal, therefore, with only 64 cases.

It is specially to be noted, that these swabs were always taken by one observer and we wish to record our debt of gratitude to Miss Smithwhite who was in charge of this section of the work. Her enthusiasm and care in collecting material, the attention devoted to keeping detailed and accurate records of each case, and the extracting of the necessary information from the field cards of the cases investigated—duty demanding much expenditure of time and labour—have greatly assisted in the prosecution of the work.

(*b*) *Inherent fallacies incidental to all bacteriological inquiries dealing with the treatment of wounds.*

In addition to the difficulties that are encountered in making an inquiry into the bacterial flora of wounds, the problem under consideration presented its own peculiar difficulties; of these the following may be cited.

(i) The diversity of methods of treatment in use both in France and in England is such that, to obtain a sufficiency of observations on which to base definite conclusions concerning any one method, would involve not months, but years of study.

(ii) It is well nigh impossible to get continuity of method in France and in England.

(iii) While a certain number of cases are evacuated to England, and find their way to hospitals in a single district in this country within a week or two after the soldiers have been wounded, the majority of wounds are not seen on this side until considerably later than this. Herein arises an almost insuperable difficulty, viz. that men whose wounds are slow to heal are sent to England, with the result that a special type of wound, showing chronic infection with a variety of organisms, both anaërobic and aërobic, is liable to predominate in a series of wounds examined in home hospitals.

(iv) Apart from these influences, there is the fact, that owing to idiosyncrasy of certain of the patients, some wounds will heal, not because of, but one might almost say in spite of, treatment; whereas others, apparently comparable to them, cannot be induced to heal under any treatment. The two cardinal points, which must not be lost sight of, are:

(*a*) That surgery is more an art than a science, therefore more depends upon the surgeon in charge of the case than on the dressing which he employs.

(*b*) That the best protection which the tissues can have against bacterial invasion due to any micro-organism, is the development of a layer of compact, healthy granulations on the raw surfaces.

Bearing these facts in mind, one could not hope to show any startling effects produced by the employment of one procedure, or of any series of procedures; so that the results given herein must be critically interpreted.

The following questions naturally arise:

(1) Does the presence of anaërobes in wounds—the presence of anaërobes in general, not the presence of certain species of anaërobes—seriously interfere with the healing of a wound?

(2) Is there any particular method of treatment commonly in use in France which leads, either to elimination of anaërobic infection or to rapid healing?

(3) Is there any method in use in home hospitals which is especially useful in attaining these ends?

(4) Is there any one surgical procedure, apart from the dressings

used, which is followed by special rapidity of repair or by elimination of anaërobes?

The problem under investigation had therefore to be examined from such standpoints, that these questions might be answered as far as possible.

(*c*) *Does the presence of anaërobes in wounds seriously interfere with the process of healing?*

In dealing with a series of cases in which swabs were taken seven days before the wound had healed sufficiently for the patient to be considered convalescent, the following results were obtained:

(*a*) No anaërobes were found in 19 instances.

(*b*) Light anaërobe infection was present in 20 instances.

(*c*) Heavy anaërobe infection was present in four instances.

(*d*) Tetanus bacilli were demonstrated in the swabs in (*c*) in three instances.

Of 43 cases which failed to heal during the period over which the investigation extended—each wound was examined for five weeks at intervals of one week—the swab taken during the last week in which the patient was under examination yielded:

(*a*) No anaërobes in 29 instances.

(*b*) Light anaërobic infection in 14 instances.

(*c*) Tetanus bacilli were present in one instance.

These figures indicate that anaërobic infection may persist in wounds up to the time of healing, and that the presence of such infection does not seriously retard the process of repair.

It must not be assumed from this statement, that an anaërobic infection is, in the opinion of the writers, of little or no importance in the pathology of the later phase of wound infection. The time, however, during which the presence of anaërobic bacilli is of special significance, is the first day or two after the injury has been received, and the variety, quite as much as the mass of the infection, is of importance at this time.

It must be appreciated too that, short of making a detailed inquiry into the flora of a large number of wounds, one cannot assume that the presence of anaërobes, even in the later phases of the process of repair, are of no significance; for the presence of bacilli of the *Vibrion septique* group, *B. Welchii* of *B. oedematiens*, and of *B. tetani* must always be a menace.

The detection however, of certain of these, is extremely difficult, and a special research would have to be devoted to the study of each, were a really satisfactory account to be given of the problem under consideration. Such detailed inquiry, in the present instance, has been therefore limited to the demonstration of *B. tetani* and of *B. Welchii*.

It is to be noted that in the case of *B. Welchii*, morphological characters were relied upon for demonstrating the presence of this bacillus—not a highly satisfactory method, but one which we were forced to adopt owing to the labour and expense which other methods would have involved.

(*d*) *Is there any method of treatment commonly used by the Army Surgeon abroad which tends to eliminate anaërobe infection?*

The wounds which were dealt with in the present series of examinations had, in the majority of instances, been treated either with flavine or with eusol, before removal to England. These wounds will therefore be grouped in three series:

(*a*) Treated with flavine.
(*b*) Treated with eusol or Carrel Dakin.
(*c*) Treated by other methods.

In considering this question, it was decided to deal only with the wounds of men who arrived in England within ten weeks of the date of wounding. If such a period be not put to the type of case considered, the difficulty of assessing the value of any procedure employed is greatly increased, owing to the number of refractory cases which would be introduced into the series.

(*a*) *Cases treated with flavine before evacuation to England.*

Twenty-two cases of the present series were treated with flavine abroad, and arrived in England within ten weeks of the receipt of injury. From them 72 swabs were examined.

35 swabs showed no anaërobic infection
32 ,, ,, light ,, ,,
5 ,, ,, heavy ,, ,,

In none of these swabs was *B. tetani* found.

(*b*) *Cases treated with eusol or Carrel Dakin before evacuation to England.*

Of 78 swabs from 22 wounds treated with eusol abroad and sent to England within the ten-week period,

26 swabs failed to give growth of anaërobes
31 ,, gave light ,, ,,
21 ,, ,, heavy ,, ,,

B. tetani appeared in eight swabs from six wounds in this series.

(*c*) *Cases treated by various methods.*

There remained 28 cases examined with the ten-week period comprising

10 treated with B.I.P.P.
7 ,, dry dressings,
7 ,, boric foments,
4 ,, saline or salt packs.

I shall consider these together, as the number of each is too small to justify their being considered separately.

Ninety-two observations were made on the 28 wounds, and the following results were obtained:

42 showed no growth of anaërobes,
39 ,, light ,, ,,
11 ,, heavy ,, ,,

B. tetani was grown from seven swabs obtained from five different wounds. Diagram VII shows these results expressed as percentages.

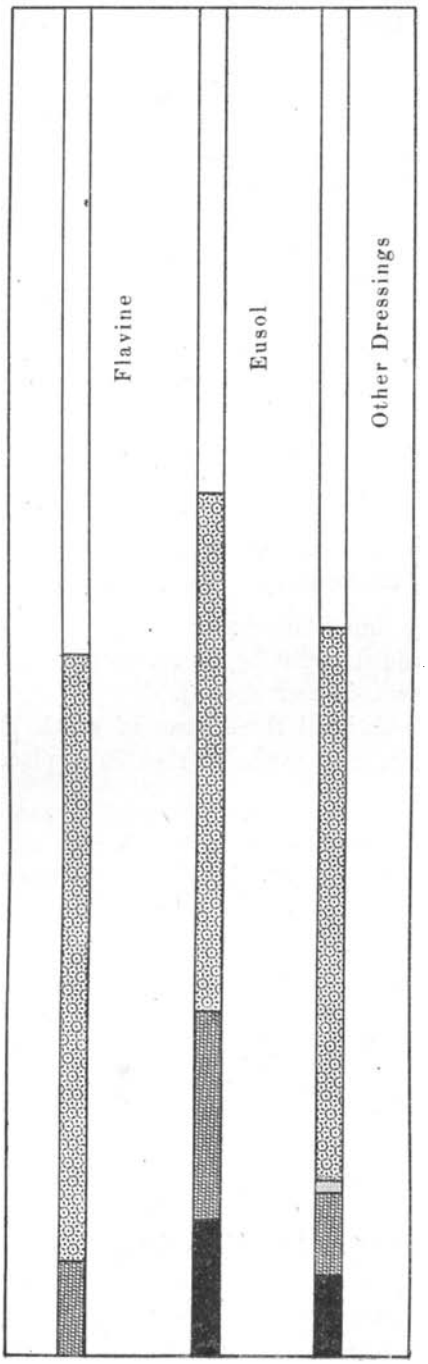

DIAGRAM VII. Influence of dressings used abroad on anaërobe infection of wounds. Results expressed as percentages of swabs examined.

= Tetani + heavy anaërobe infection. = Tetani + light anaërobe infection.

= Heavy anaërobe infection. = Light anaërobe infection.

= No anaërobe infection.

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The dark columns refer to heavy anaërobe infection, the light to light anaërobe infection. The black columns indicate that *B. tetani* was found along with heavy infections of other anaërobes, and the hatched column that *B. tetani* was present with light concomitant anaërobe infection.

It is not easy to comment on the results shown in the above diagram, for they may well give a false impression, owing to the fact that 11 of the wounds in the flavine series had been excised, while only six of those of the eusol series, and five of those in which other dressings had been used, were so treated.

Both the low rate of "heavy anaërobe infection" and the absence of tetanus bacilli in those wounds originally treated by flavine, appear to suggest that that reagent is suitable for primary dressings. The results may, however, mean that excision of the wounds was, in the instances quoted, the most important factor in eliminating infection.

This subject will be again considered in dealing with the "time factor" in the healing of excised wounds, as compared with that of non-excised wounds.

In Diagrams XIII and XIV (pp. 191, 192) are graphically shown the results of each examination of the swabs dealt with above.

(*e*) *Influence of dressings in use in the Home Hospitals on the degree and persistence of anaërobic infection of wounds.*

In this series, the arbitrary time limit of ten weeks was again adhered to, in order that the results obtained might be, as far as possible, comparable with those dealing with the methods used abroad.

The series comprises an additional three cases in which the treatment abroad was not recorded on the field cards, so that 75 in place of 72 cases are herein considered.

(*a*) *Cases treated by boric fomentations.*

It was surprising to find that boric fomentation was the favourite method of treatment in the hospitals from which the material was obtained, and of cases so treated there were 35 involving 123 observations.

55 swabs failed to give growth of anaërobes,
49 ,, gave light ,, ,,
19 ,, ,, heavy ,, ,,

B. tetani was obtained from 11 swabs in seven cases.

(*b*) *Cases treated by eusol.*

Of cases treated by eusol or the Carrel Dakin method there are 22, involving 58 observations.

27 showed no anaërobe infection,
23 ,, light ,, ,,
8 ,, heavy ,, ,,

B. tetani was obtained from three swabs in three cases.

(*c*) *Cases treated by other methods.*

Eighteen cases of the series were treated by other methods (comprising 13 in which saline was used), in which a variety of procedures had been employed.

These involved 71 observations, giving the following results.

30 swabs failed to give growth of anaërobes,
30 ,, showed light anaërobe infection,
11 ,, heavy ,, ,,

B. tetani was found in three instances from three cases.

These results are summarised in Diagram VIII, p. 180.

These results are instructive, for they show in a remarkable manner, that no particular dressing can be recommended as likely to produce marked diminution in the anaërobe flora of wounds during the process of repair.

Experimental evidence bears this out—thus, Brilliant Green, which in certain low concentrations will markedly inhibit the growth of bacteria, including the anaërobes, in broth or in serum, is much reduced in its inhibitory activity if a piece of fresh living tissue be added to the cultures.

The conditions obtaining in cultures in fluid media enriched by the addition of fresh tissue, more closely approximate the conditions of a wound, than do cultures which are not so enriched.

It is probable that this inhibition of antiseptic activity demonstrated in culture tubes is multiplied manifold in wounds, owing to the large surface of granulation to which the reagent is exposed.

All that can be hoped for then, from the use of any antiseptic so far employed, is, that it will reduce mass infection of wound exudates; it will not, however, eliminate infection.

As a mild degree of anaërobe infection is quite compatible, so far as can be seen, with unretarded progress of healing; and, as we cannot hope to eliminate the anaërobes by the use of any antiseptic, the question at issue must be dealt with from another point of view.

The details of the examination of each swab are given in Diagrams XV and XVI (pp. 193, 194) at the end of this section.

(*f*) *Rapidity with which healing takes place under various dressings.*

So long as a wound remains open it is, to a greater or less degree, susceptible to bacterial invasion of all kinds. If then, any particular procedure can be shown to cause rapid healing of wounds, such is to be recommended, both from the standpoint of surgery and from that of economics.

Three points of view have to be considered in dealing with this question.

(*a*) The nature of the dressing used abroad.

(*b*) The nature of the dressing in home hospitals.

(*c*) The wounds treated by excision must be contrasted with those that have not been excised.

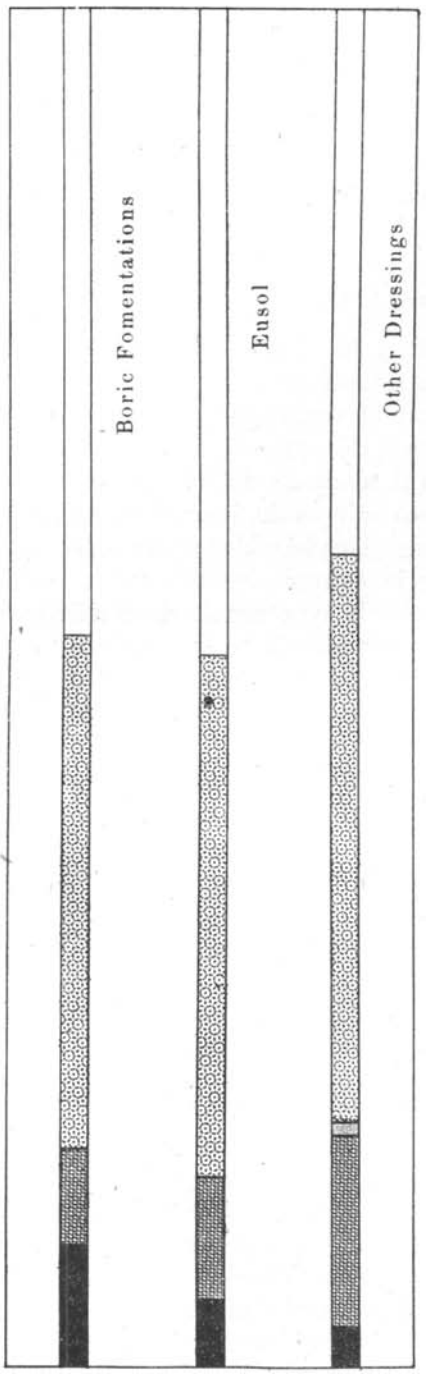

DIAGRAM VIII. Influence of dressings used in Home Hospitals on anaërobe infection. Results expressed as percentages of swabs examined.

= Tetani + heavy anaërobe infection.
= Tetani + light anaërobe infection.
= Heavy anaërobe infection.
= Light anaërobe infection.
= No anaërobe infection.

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(*f'*) *Influence of dressings used abroad upon the rate of healing.*

1. *Flavine.*

Twenty-one wounds fulfilling the conditions arbitrarily laid down concerning evacuation to England within a ten-week period, were treated with flavine abroad. Of these 14 recovered, the recoveries being distributed thus:

Patients examined in England within	Number of wounds examined	Number which healed
1 week	2	2
2 weeks	8	6
3 ,,	4	4
4 ,,	2	0
5 ,,	1	0
6 ,,	1	0
7 ,,	1	0
8 ,,	0	0
9 ,,	2	2
10 ,,	0	0

2. *Eusol.*

Twenty-two wounds receiving early treatment with this reagent behaved as follows:

Patients examined in England within	Number of wounds examined	Number which healed
1 week	3	2
2 weeks	3	1
3 ,,	3	1
4 ,,	2	1
5 ,,	3	2
6 ,,	2	0
7 ,,	1	0
8 ,,	1	1
9 ,,	1	0
10 ,,	3	2

3. *Other methods.*

In the case of 26 wounds which had been treated abroad by other methods, the following results were obtained:

Patients examined in England within	Number of wounds examined	Number which healed
1 week	4	3
2 weeks	8	5
3 ,,	5	1
4 ,,	2	2
5 ,,	0	0
6 ,,	1	0
7 ,,	2	0
8 ,,	2	2
9 ,,	0	0
10 ,,	2	0

These results are summarised in Diagram IX, in which both the recovery rate and the time when recovery occurred are graphically shown. For the purposes of comparison the figures are reduced to a common denominator.

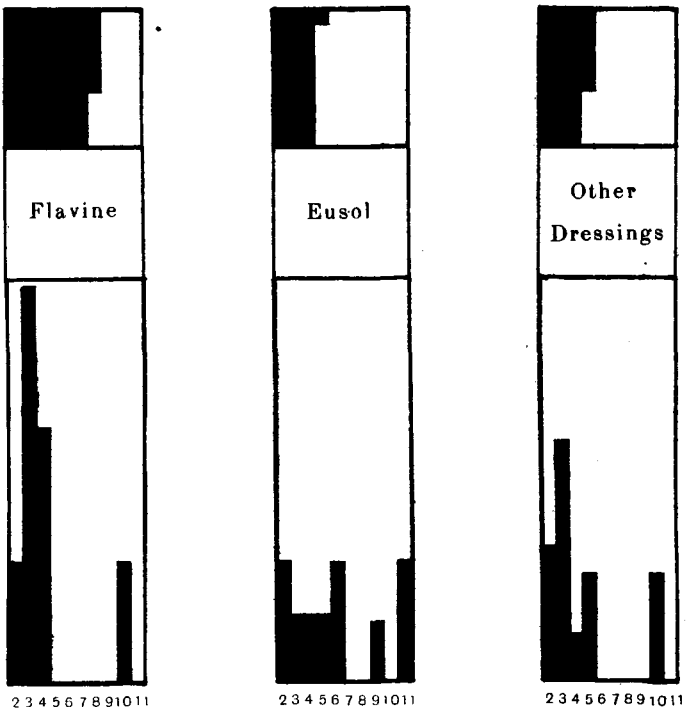

Diagram IX. The upper portion of the diagram indicates the percentage recovery with each dressing. The lower indicates the wounds which recovered. Figures relate to week in which recovered cases were first examined in England. An arbitrary time limit of five weeks from first examination was taken as period for recovery to take place.

These results appear to indicate that flavine is the most valuable dressing of those considered in the present series, both as regards the number of recoveries and the rapidity of recovery.

It must be noted, however, that there is a preponderance of excised wounds in the flavine series.

(*f''*) *Influence of dressings used in Home Hospitals.*

When considered from the standpoint of dressings used in Home Hospitals, the following results appear:

1. *Boric foments.*

Thirty-six wounds were treated by boric fomentations, with 12 recoveries, distributed as follows:

Patients examined in England within	Number of wounds examined	Number which healed
1 week	5	2
2 weeks	7	4
3 ,,	7	3
4 ,,	3	1
5	3	0
6 ,,	2	0
7 ,,	3	0
8 ,,	0	0
9 ,,	6	2
10 ,,	0	0

2. *Eusol.*

Twenty-two wounds were treated with eusol, giving 15 recoveries, distributed as follows:

Patients examined in England within	Number of wounds examined	Number which healed
1 week	4	4
2 weeks	5	4
3 ,,	3	2
4 ,,	1	1
5 ,,	2	1
6 ,,	1	1
7 ,,	4	2
8 ,,	0	0
9 ,,	0	0
10 ,,	1	0

3. *Other methods.*

Eighteen wounds were treated by other methods—with nine recoveries, distributed as follows:

Patients examined in England within	Number of wounds examined	Number which healed
1 week	0	0
2 weeks	5	4
3 ,,	8	2
4 ,,	2	1
5 ,,	1	1
6 ,,	0	0
7 ,,	1	1
8 ,,	0	0
9 ,,	0	0
10 ,,	1	0

Note. Of the series treated by boric foments four had been excised. Of those treated with eusol nine and of those by other methods three.

These results are summarised in Diagram X, p. 184, which shows graphically both the recovery rate and the time when recovery took place.

The results summarised in Diagram X suggest that the cases treated with eusol in Home Hospitals heal more rapidly than do those treated by other methods. Here again, however, there is seen a preponderance of the excised wounds in the series.

The results then do not give information as to which dressings of those examined could be recommended for use in England, as being of special value in stimulating healing.

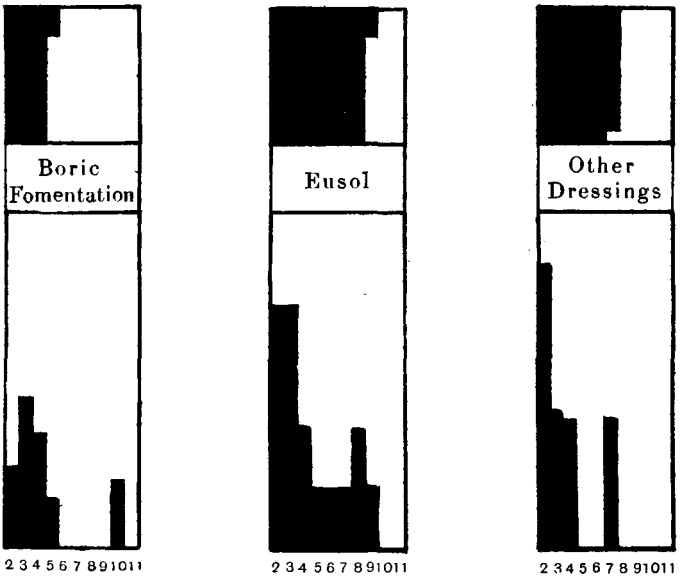

DIAGRAM X. The upper portion of the diagram indicates the percentage recovery with each dressing. The lower indicates the wounds which recovered. Figures relate to week in which recovered cases were first examined in England. An arbitrary time limit of five weeks from first examination was taken as period for recovery to take place.

(*g*) *Influence of excision.*

Is there any one surgical procedure, apart from the dressings employed, which leads to the rapid healing of wounds?

If excised wounds be contrasted with those not so treated, it is seen that within the arbitrary period of ten weeks, 16 out of 26 excised wounds had progressed sufficiently for the patients to be considered convalescent. While of the non-excised wounds, 51 in number, 24 became convalescent. These findings are summarised in Diagram XI, in which are indicated both the recovery rate, and the time within which recovery occurred.

This diagram indicates:

(*a*) That the recovery rate is higher in the *excised* than in the *non-excised* wounds.

(*b*) That the distribution of the recoveries in point of time is more uniform in the *excised* than in the *non-excised* wounds. This latter point appears to the writers to be a matter of some importance, as it suggests, that a more diverse type of wound heals within a given period after excision, than is the case when excision is not practised. This really means, that in the

case of the *non-excised* wounds, unless healing occurs within the first three or four weeks, the convalescence may be protracted.

The details of the examination of swabs from *excised wounds*, irrespective of the dressing used, are shown graphically in Diagram XVII, p. 195.

Before proceeding to the next sub-section of the investigation, dealing with wounds, the examination of which was commenced after the expiry of

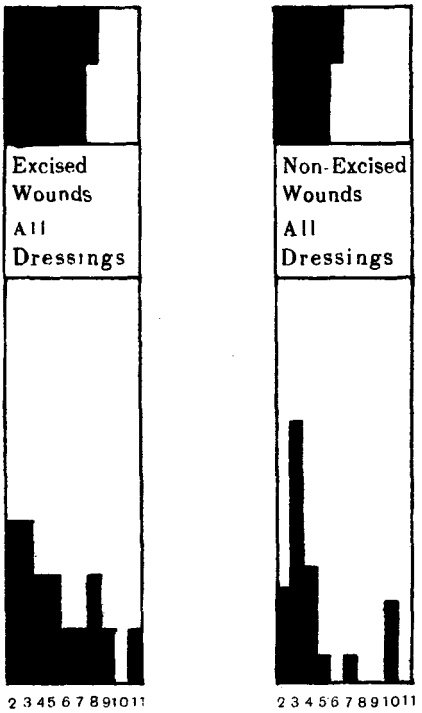

DIAGRAM XI. The upper portion of the diagram indicates the percentage recovery in each instant. The lower indicates those wounds which recovered. Figures relate to week in which recovered cases were first examined in England. An arbitrary time limit of five weeks from first examination was taken as period for recovery to take place.

the ten-week period, the question of the influence which anaërobes have upon healing, and also the influence which various dressings exert upon anaërobe infection, may be summarised thus:

(i) A mild degree of anaërobe infection does not prevent, and does not apparently even retard, the process of healing.

(ii) Wounds treated with flavine in France have, on the whole, a lesser degree of infection with anaërobes, than those treated by other methods. The fallacy due to the preponderance of *excised* wounds so treated, renders it difficult to offer an opinion as to whether flavine or excision has been the factor producing the desired result.

(iii) None of the treatments used in home hospitals, which we were in a position to investigate, eliminate anaërobe infection with especial rapidity.

(iv) As regards the rapidity with which the wounds healed, the best results were obtained in those treated with flavine abroad and with eusol in England. It is to be noted that a preponderance of *excised* wounds in both instances renders difficult the assessment of these results.

(v) On contrasting *excised* with *non-excised* wounds, it is found, that the recovery rate is higher and more uniform in wounds which have been *excised* than in those which have not been so treated.

(*h*) *Examination of wounds of long standing.*

In addition to the series that has already been considered, 12 wounds were examined in men who arrived in England between the 10th and 15th weeks after reception of the injury. This involved the examination of 37 swabs.

No anaërobes found in 23 instances,
Light anaërobic infection in 10 instances,
Heavy „ „ 4 „

B. tetani was found in seven swabs from five cases.

Finally, as a matter of scientific interest, it was decided to examine some wounds of very long standing.

(*a*) Wound examined during the 17th and 18th weeks gave one swab which showed a heavy growth, and one which failed to give growth of anaërobes.

(*b*) Wound examined from the 18th to the 23rd week gave three swabs producing a heavy growth of anaërobes, and two which showed no growth of such organisms.

(*c*) Wound examined from the 22nd to the 25th week gave two swabs showing heavy anaërobic infection, one with light infection, while in two anaërobes failed to grow.

(*d*) Wound examined from the 23rd to the 27th week gave three swabs with light anaërobe infection and two with none.

(*e*) Wound examined from the 30th to the 33rd week gave no growth of anaërobes.

(*f*) Wound examined during the 32nd and 33rd weeks gave one swab with a light anaërobe infection and one showed no anaërobe infection.

(*g*) Wound examined from the 35th to the 38th week, one swab showed heavy growth of anaërobes and three showed no growth of anaërobes.

(*h*) Wound examined from the 37th to the 40th week all four swabs failed to give growth of anaërobes.

(*i*) Wound examined from the 38th to the 41st week gave four successive swabs showing heavy anaërobe infection.

(*j*) Wound examined from the 42nd to the 45th week gave three swabs showing light anaërobe infection and one showing none.

(*k*) Wound examined from the 43rd to the 47th week gave five successive swabs containing *B. tetani*, three being accompanied by heavy and two with light anaërobe infection.

(*l*) Wound examined from the 44th to the 47th week gave one swab with light anaërobe infection and three with none.

If then the results obtained in examining wounds between the 15th and 50th weeks after receipt of injury be summarised, the following results are obtained:

24 wounds were examined involving the examination of 82 swabs.
In 46 instances no anaërobes were found,
„ 22 „ light anaërobe infection was found,
„ 14 „ heavy „ „ „

B. tetani was obtained in 12 swabs from six cases.

On six occasions *B. tetani* was found along with heavy anaërobe infection and on the other occasions with light anaërobe infection.

It seems at first sight remarkable that these results are so bad, and compare unfavourably with any that have gone before, but the wounds considered are practically picked refractory cases and therefore occupy a category by themselves.

There are, in addition, two especially interesting observations:

(i) Five swabs were taken from a case between 104th and 108th weeks from date of wounding, three out of the five swabs were heavily infected with anaërobes, two out of the three containing *B. tetani*.

(ii) Five swabs were also taken from a case between the 106th and 130th weeks after reception of the injury, two gave a heavy growth of anaërobes *B. tetani* being present in large numbers in the cultures, one gave a light growth of anaërobes, and two gave no growth of these organisms.

These observations call attention to the remarkably long periods during which *B. tetani* and other anaërobes may persist in wounds. The importance of the observations is, that they indicate how necessary it is to administer a prophylactic dose of antitoxin the day before any operation for cosmetic or other purpose is performed at the site of an old wound. It would be advisable, too, to give, if possible, anti-gas-gangrene serum along with the prophylactic tetanus antitoxic serum.

(*i*) *Examination for presence of* B. tetani *in a series of* 100 *wounds.*

The tetanus bacilli obtained from 100 consecutive wounds of men showing no evidence of tetanus are tabulated on p. 188.

Commenting on these results, it is seen, that 14 cultures elaborating spasm-producing toxin were obtained from 100 consecutive wounds. In carrying out the toxin tests 0·2 c.c. was the largest quantity employed, when mice were the experimental animals used.

In four instances, while the toxin experiments were negative, typical

No.	Animal experiment	Agglutination	Since wounding	Where wounded
1.	Toxin experiment positive	Type I	7 days	Ypres
2.	,, ,, ,,	,,	7 ,,	Ypres
3.	,, ,, ,,	,,	22 ,,	Ypres
4.	,, ,, negative Infection experiment positive	,,	24 ,,	Albert
5.	Toxin experiment ,,	,,	25 ,,	Villers Bretonneux
6.	,, ,, ,,	,,	30 ,,	Mericourt
7.	,, ,, negative Infection experiment positive	,,	32 ,,	Cambrai
8.	Toxin experiment negative Infection experiment positive	,,	35 ,,	Cambrai
9.	Toxin experiment negative	,,	39 ,,	Cambrai
	Infection experiment positive	,,	39 ,,	Cambrai
10.	Toxin experiment ,,	,,	64 ,,	Battersea
11.	,, ,, ,,	,,	112 ,,	Ypres
12.	,, ,, ,,	,,	130 ,,	Cambrai
13.	See Footnote 1	,,	733 ,,	Hohenzollern Redoubt
14.	Toxin experiment positive	,,	860 ,,	Poelcapelle
15.	,, ,, ,,	,,	882 ,,	Cambrai
16.	,, ,, ,,	Type II	15 ,,	Bapaume
17.	,, ,, ,,	,,	138 ,,	Passchendaele
18.	See Footnote 2	,,	100 ,,	Cambrai
19.	,, ,,	,,	54 ,,	Monchy
20.	Toxin experiment positive	Type III	16 ,,	Orvillers
21.	,, ,, ,,	Type IV	305 ,,	Hermies

Footnote 1. With reference to No. 13 toxin experiments were negative, and when an attempt was made to carry out the infection experiment, the animals died from gas gangrene.

Footnote 2. Cultures 18 and 19. Culture 18, which agglutinated in presence of Type II serum, was lost before the animal experiments were completed. Culture 19 is still under observation.

tetanus infection could be produced when washed cultures were injected together with a tissue debilitant. Therefore, in at least 18 per cent. of the present series of wounds, tetanus bacilli could be recovered.

This figure is considerably higher than any previous investigations would have led one to expect. The reason for this somewhat high figure is, that unless wounds be repeatedly examined, tetanus bacilli if present in small numbers may be missed; just as throat swabs, in cases of diphtheria, may give negative results owing to the operator having failed to swab that area of the throat in which *B. diphtheriae* is present.

Diagrams XIII, XIV, XV, XVI and XVII which give the result of the examination of each swab graphically illustrate this point.

(*j*) *Examination for presence of organisms having the morphological characters of* B. Welchii *in a series of* 100 *wounds.*

In the series of wounds under consideration the presence of organisms having the morphological appearance of *B. Welchii* was also noted, and an attempt was made to determine whether any of the dressings commonly

used, either at home or abroad, tended rapidly to eliminate this organism from wound exudates.

From this inquiry, the following conclusion was drawn: "That no dressing among those investigated could be especially recommended as likely to produce rapid elimination of *B. Welchii*."

As the details of the examination constitute what is virtually a repetition of the findings already set forth in connection with the inquiry into the influence which various dressings exert upon the reduction of all anaërobic infections, they will not be dealt with *in extenso*.

The results of the examinations call attention, however, to a marked difference between *excised* and *non-excised* wounds in this connection. While there is but little difference between the percentages of *excised* and *non-excised* wounds, the exudates of which contain *B. Welchii*, there is a marked difference between the two classes in respect of the period over which infection with *B. Welchii* can be demonstrated. This period is much shorter in the case of *excised* wounds than in the case of *non-excised* wounds.

The actual findings are as follows:

On a series of 100 wounds 46 were found to contain *B. Welchii*.

(*a*) In four wounds—two excised, two non-excised—*B. Welchii* disappeared before the second week.

(*b*) In eight wounds—five excised, three non-excised—*B. Welchii* disappeared before the third week.

(*c*) In six wounds—one excised, five non-excised—*B. Welchii* disappeared before the fourth week.

(*d*) In three non-excised wounds, it disappeared before the fifth week.

(*e*) In four wounds—three excised, one non-excised—it disappeared before the sixth week.

(*f*) In two wounds—one excised, one non-excised—it disappeared before the seventh week.

(*g*) Thereafter, only non-excised wounds contained *B. Welchii*. In one this organism disappeared before the 8th week, three before the 9th week, one before the 11th, four before the 13th, one before the 15th, one before the 16th, two before the 17th; and five wounds were shown to contain *B. Welchii* between the 27th and 47th weeks after infliction of the injury. These facts are set forth diagrammatically in the following figure.

Note. Attention is called to the fact, that in many of the instances quoted above, the number of organisms present in the cultures which had the morphological appearance of *B. Welchii* was small. It is probable that, without careful and repeated examination of the growths, the presence of this bacillus would not have been appreciated.

These findings are in full agreement with those which were obtained in making inquiry into the influence which various dressings and surgical procedures exert upon all anaërobic infections of wounds.

They are especially valuable, in that they show fairly conclusively, "*that excision does not eliminate infection with the anaërobic bacilli but it removes*

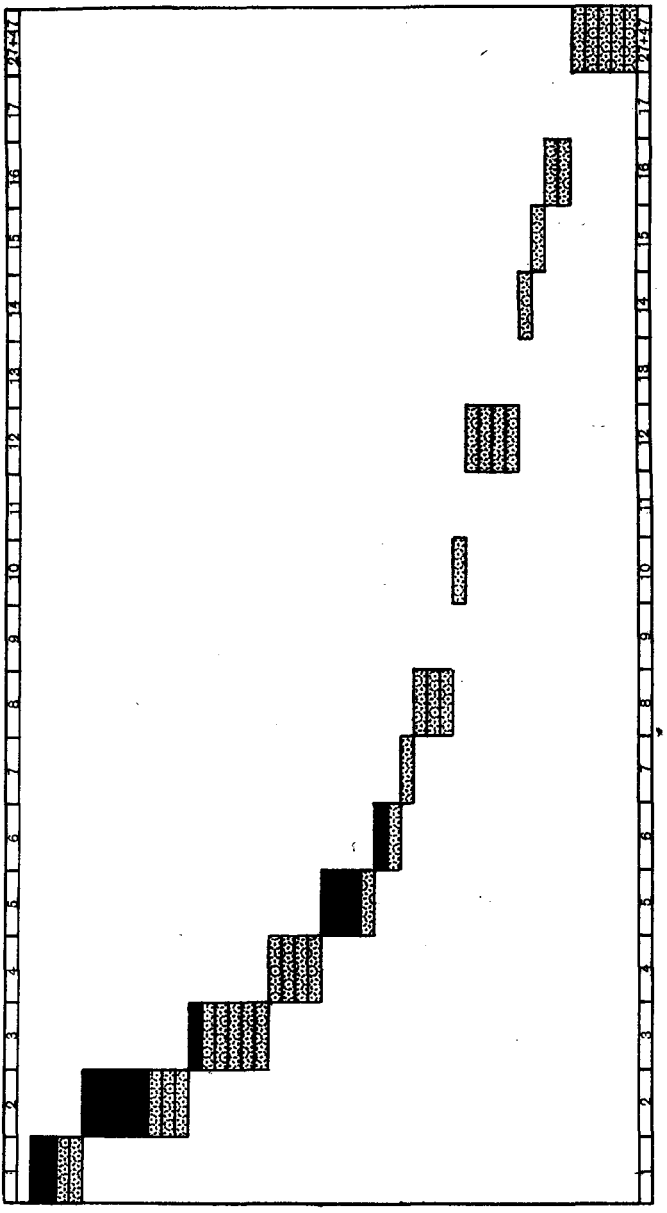

DIAGRAM XII. This figure shows the number of wounds in which *B. Welchii* was present and indicates the last swab in which it could be demonstrated in each instance.

= In excised wounds.

= In non-excised wounds.

Figures = Weeks since reception of injury.

Total number of excised wounds = 27, *Welchii* present in 12 = 44 %.

Total number of non-excised wounds = 73, *Welchii* present in 34 = 46 %.

those conditions which enhance the danger arising from the presence of these organisms."

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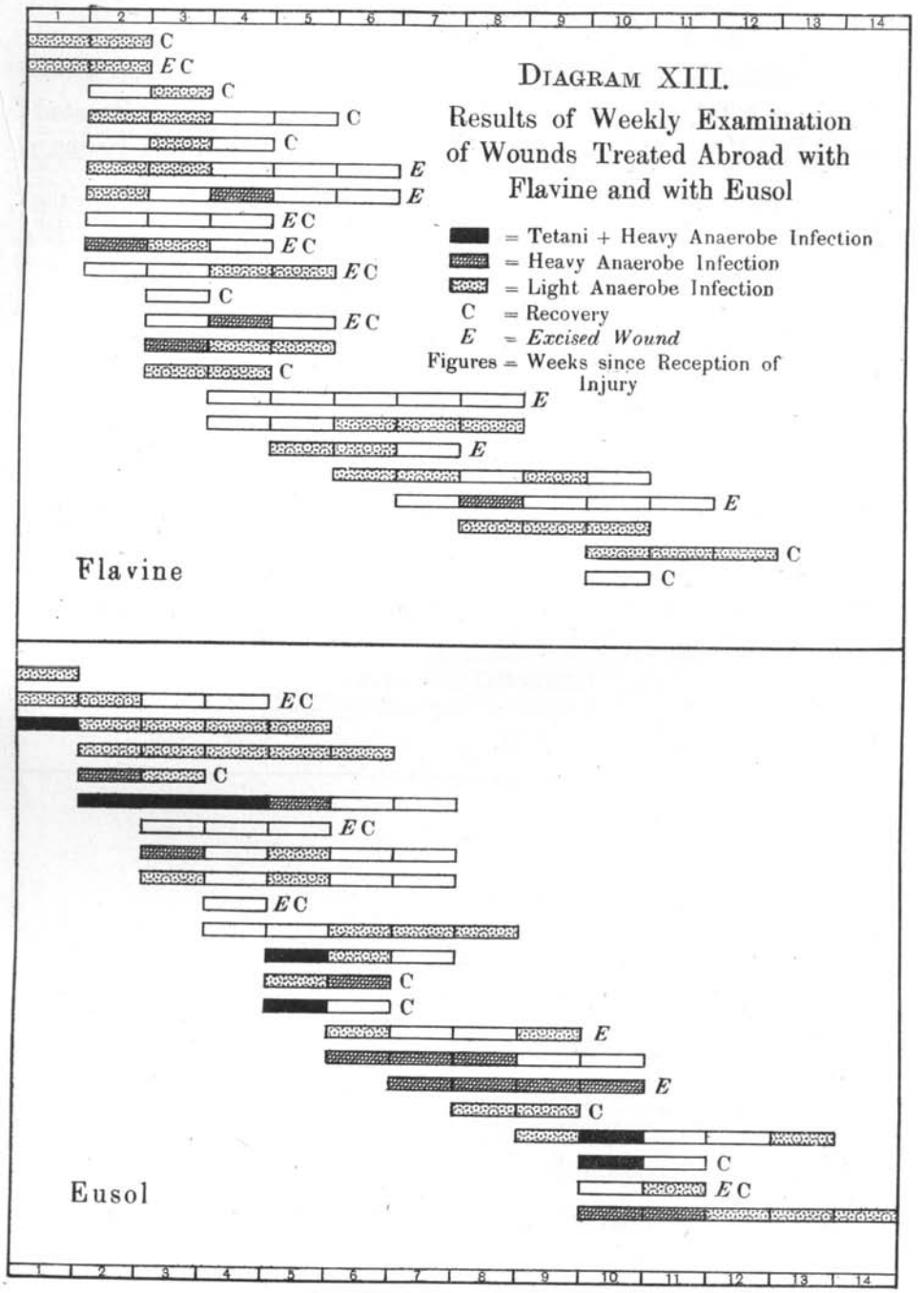

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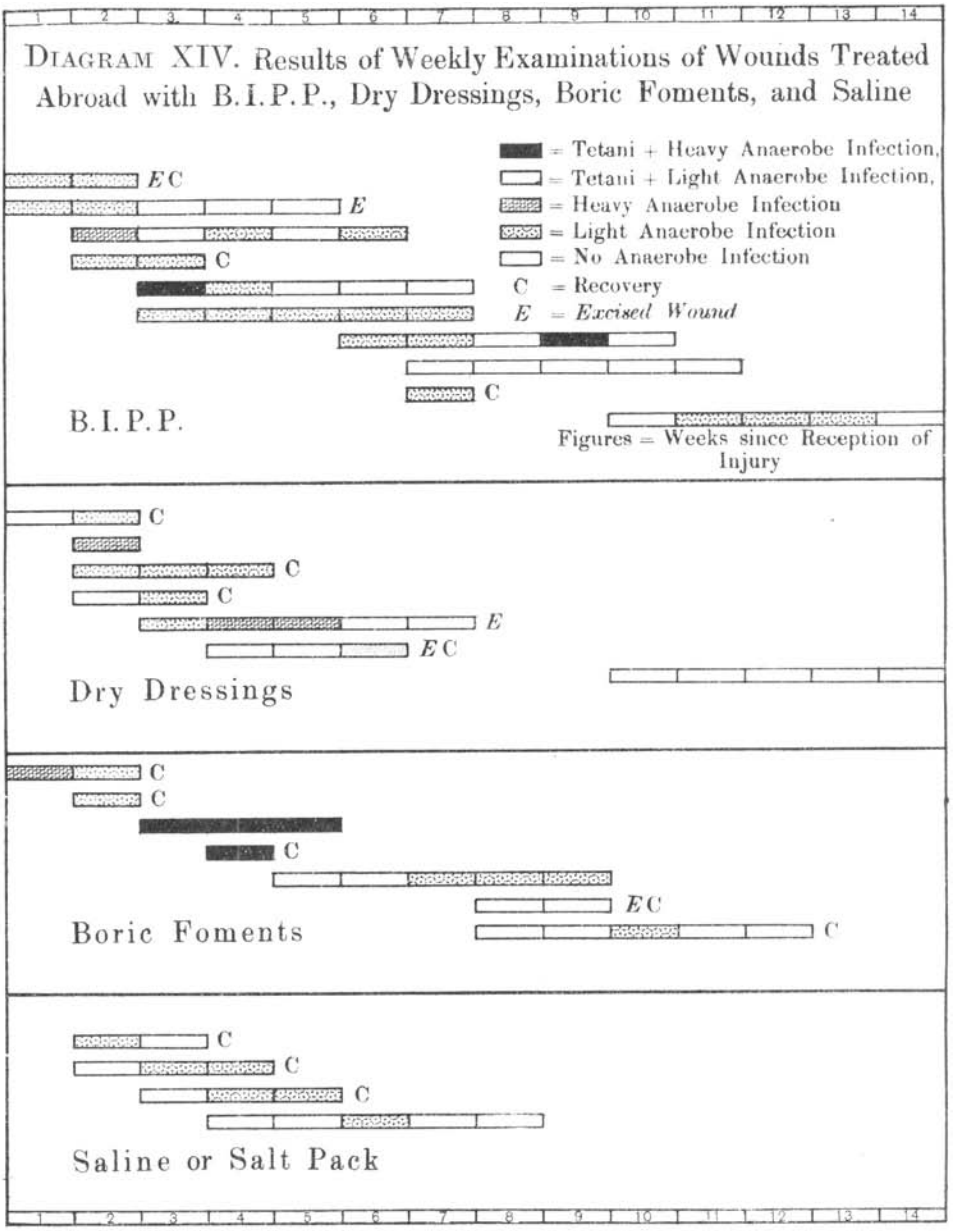

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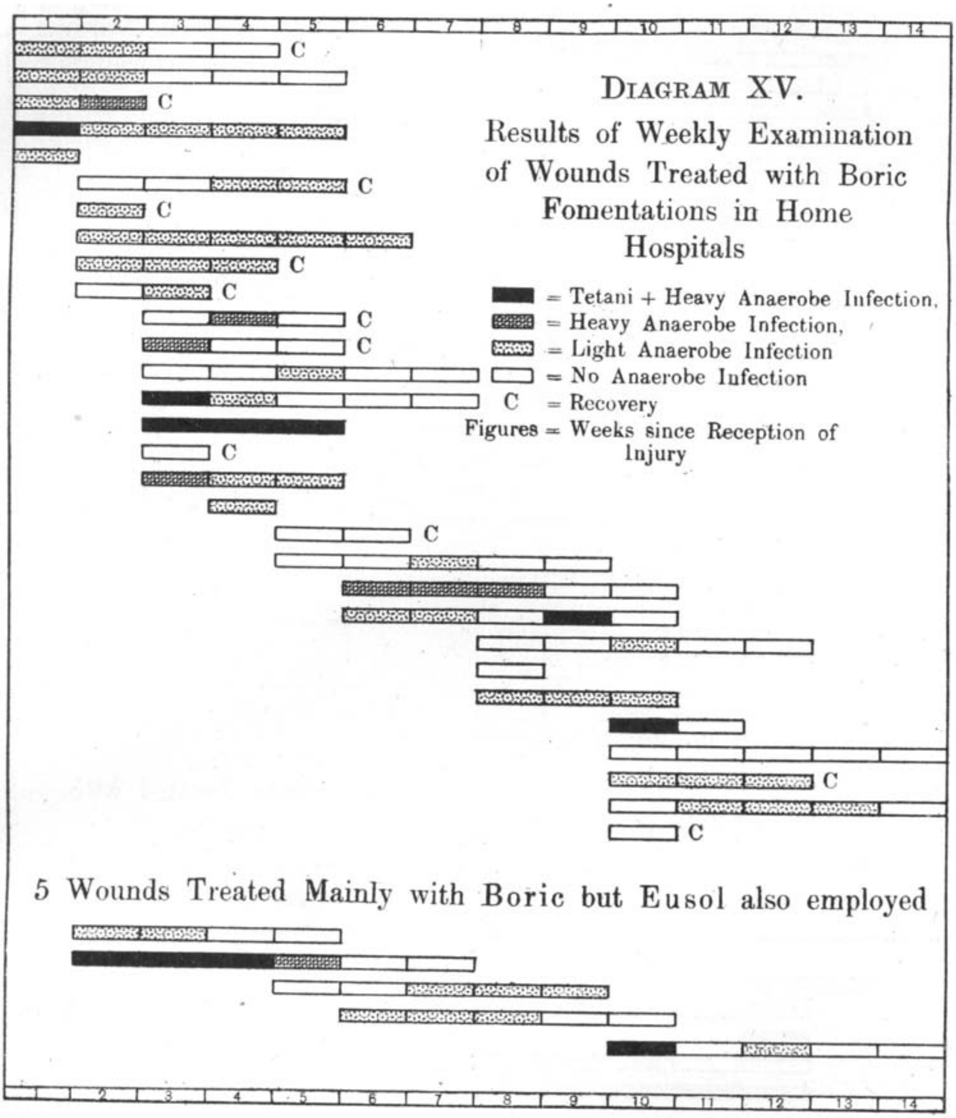

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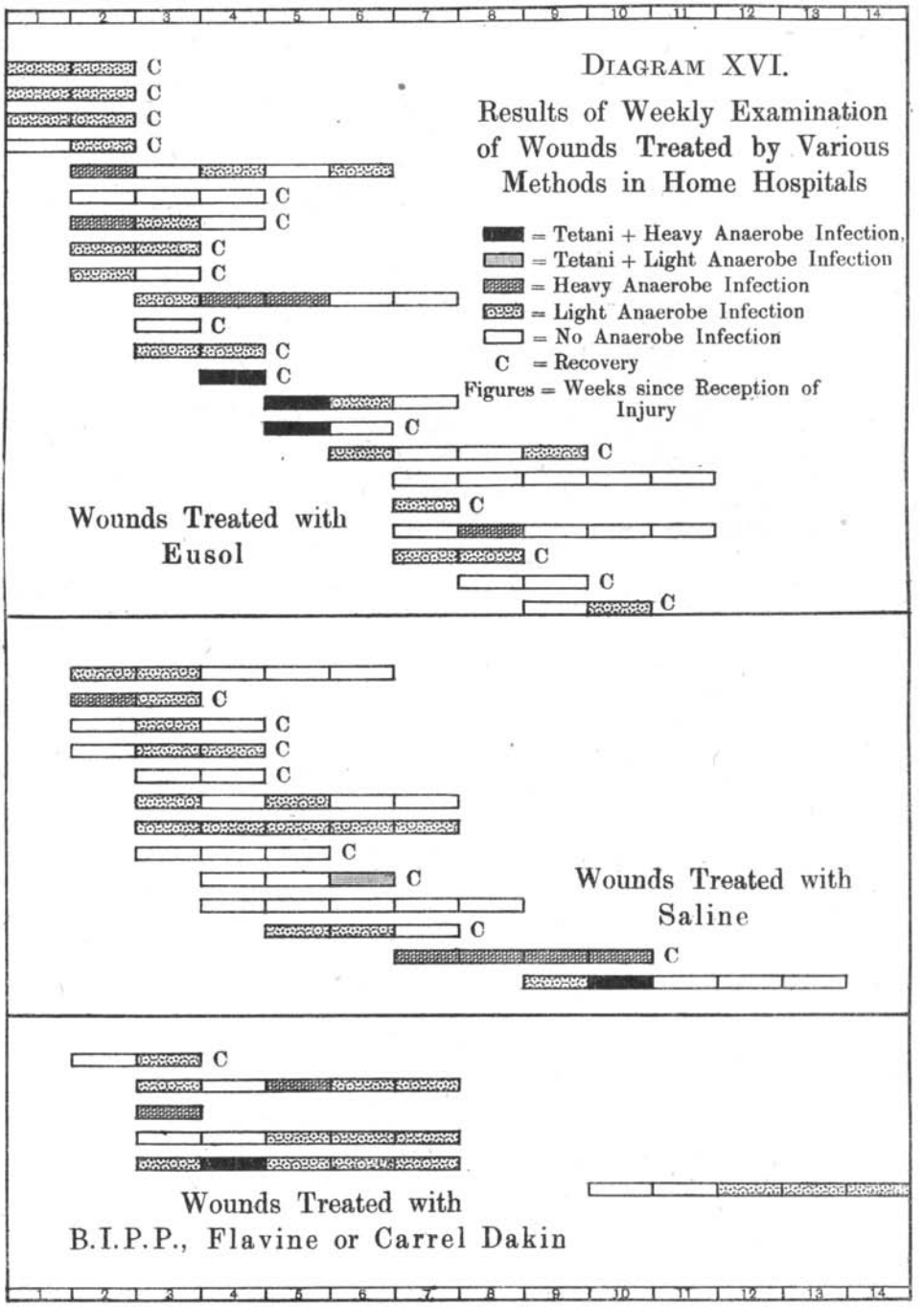

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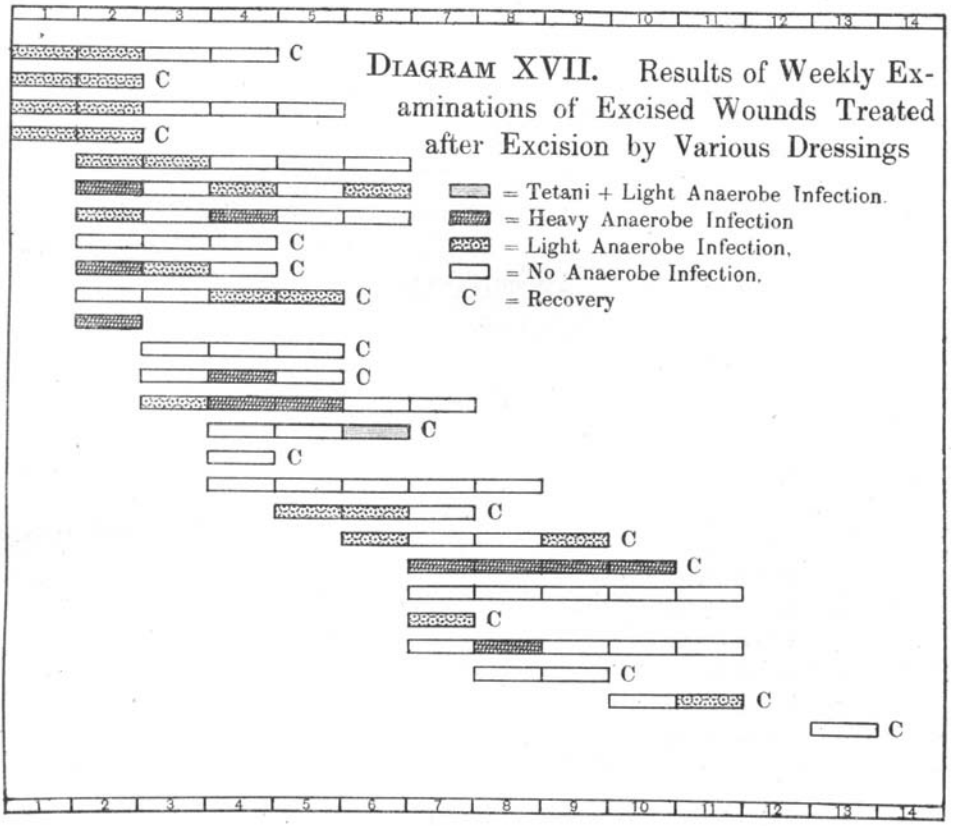
CONCLUSIONS FROM SECTION VI, pp. 172–195.

1. No one dressing, of those dealt with in the present investigation, can be especially recommended as being valuable for the elimination of anaërobic micro-organisms in general from wound exudates.

2. Neither can any one dressing be recommended as especially valuable in eliminating *B. tetani* or *B. Welchii*.

3. The presence of a mild degree of anaërobe infection in wounds does not necessarily prevent, or even retard, the process of healing.

4. Excised wounds heal more rapidly than do non-excised wounds, and therefore by excision the danger period is reduced during which infection with either *B. tetani* or the gas gangrene bacilli may be a menace.



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SECTION VII.

CAN AGGLUTINATION OF STOCK EMULSIONS OF *B. TETANI* BY THE SERUM OF PATIENTS SUFFERING FROM EARLY TETANUS BE EMPLOYED AS A METHOD FOR DIAGNOSIS AND FOR DETERMINING "TYPE OF INFECTION" IN TETANUS?

Serum from over 60 cases of tetanus was used for agglutinating stock emulsions of the 4 Type bacilli. At the same time a number of normal sera were tested in the same way.

The result showed that certain normal sera may agglutinate tetanus bacilli of one or other Type in dilutions up to 1/80. None of the sera from cases of the disease could be definitely demonstrated to be of much higher titre; and further, in those cases in which the infecting organism was typed, no relationship could be shown between the Type of the infecting organism and the agglutination reaction performed with the patient's serum. One had to conclude, therefore, that agglutination could not be employed for making a "preclinical" diagnosis of tetanus, nor could it be used for determining to which serological Type of the bacillus the organism belonged which was responsible for the causation of the disease in each case.

SECTION VIII.

RELATION OF SEROLOGICAL TYPE OF BACILLUS ISOLATED, TO HAEMAGGLUTINATING TYPE OF MEN FROM WHOM THE BACILLUS WAS OBTAINED.

As a matter of scientific interest, the haemagglutinating property of these sera was, where possible, determined. The reason for undertaking this inquiry was, that it might be suggested, that the serological Type of the organism depended upon its being parasitic on individuals belonging to a particular haemagglutinating group of men; for instance, the Type I bacillus might evolve as a result of its being parasitic upon an individual of haemagglutinin Group II. Type III bacillus might result from parasitism on an individual belonging to another haemagglutinating group, etc. Such a relationship could not be demonstrated; for example, of four individuals from whom Type III bacilli were isolated, two belonged to haemagglutinin Group II, one to Group III, and one to Group IV.

SECTION IX.

A NOTE ON TWO CASES OF ABDOMINAL TETANUS.

The two following cases are of especial interest, in that tetanus developed after the performance of an abdominal operation, and the source of infection in each case was undoubtedly the intestine.

Case I.

A man, who had been wounded in France one year previously, was taken ill with appendicitis; some days after the operation tetanus developed with a fatal result.

Examination of material from the wound in the abdominal wall, from the stump of the appendix, and from the faeces in the descending colon, all yielded a growth of tetanus bacilli of serological Type I and all virulent for animals.

Case II.

In this case, which was forwarded to me unofficially, the victim of the disease was a girl of 4½. The history of the case was as follows:

Patient was admitted suffering from intussusception—a laparotomy was performed the same day and the intussusception was reduced. Five days later tetanus developed and terminated fatally. Material from the wound and faeces were both submitted for examination. Type III tetanus bacilli were found in both.

The main interest of the second case is that as the gut had not been opened, therefore the bacillus must have been in the gut, and the cause of the *infection* was probably the tissue debility produced by the intussusception.

SECTION X.

PRESENCE OF *B. TETANI* IN THE FAECES OF MEN RETURNED FROM OVERSEAS COMPARED WITH THOSE FROM CIVILIAN FAECES.

Up to the present 21 specimens of faeces from men returned from overseas have been examined, and in seven instances organisms were obtained which agglutinated with one or other of the Type agglutinating sera. Of these, four were Type I bacilli, two were Type II and one was Type III. Of 31 specimens of faeces from civilians five gave cultures which agglutinated with Type sera. All proved to be Type I. It is to be noted that the investigation of these faeces was not so complete as was that of cultures obtained either from the wounds of men suffering from tetanus or from wounds of men showing no clinical evidence of tetanus.

The inquiry, therefore, makes no claim to accuracy, and the results obtained are mainly of academic interest.

The reason for conducting this inquiry was, that a number of cases of abdominal tetanus in soldiers had been reported to Major-General Sir David Bruce, K.C.B., F.R.S. The aggregate of these cases was considerable. Two explanations of their occurrence had to be considered:

(i) It might be that owing to the machinery for collecting information *re* tetanus being adequate, these cases were returned among those due to tetanus infection of war wounds. Seen together the number appeared to be

relatively large, but without consulting the Registrar General's returns, it could not be assumed that the number was larger than in times of peace.

(ii) It might be, on the other hand, that the conditions of active service leading to contamination of food stuffs, etc., with earth, resulted in sufficient unconscious geophagy to alter the flora of the gut, and to increase the number of bacillus tetani. The figures quoted are insufficient to demonstrate that the latter explanation is correct, and it is highly probable that the larger number of cases of abdominal tetanus among soldiers is apparent rather than real.

Nevertheless in view of the findings reported in this section, and in view also of the findings previously published by Pizzini, it might be advisable to err on the side of safety, and administer a prophylactic dose of antitoxin before performing an abdominal operation involving section of the gut, especially when such operations are performed on recently returned men.

SYNOPSIS AND GENERAL CONCLUSIONS.

I. In Sections I, II, and III, are described the methods employed, and the results obtained, in making a systematic investigation of the prevalence of the various serological Types of *B. tetani*, both in wounds of men suffering from tetanus and in those of men showing no evidence of that disease.

This inquiry shows:

1. That the mortality among inoculated men from infection due to tetanus bacilli belonging to serological Type I is lower than that from infection with either Type II or Type III.

2. If the observations be limited to those cases in which the incubation period is 14 days or less, the mortality from infection due to Types II and III is higher than when Type I is the organism responsible for the causation of the disease.

3. If observations on the incidence of cases due to the various Types of *B. tetani* be considered from the same point of view, relatively more cases of Type II and Type III infection declare themselves within a period of 14 days than Type I.

4. From an unexpectedly large percentage—20 per cent.—of wounds of men who show no evidence of tetanus, *B. tetani* can be recovered at some period during the process of repair.

5. Of tetanus bacilli obtained from such "indifferent" wounds the majority conform to serological Type I.

These facts are susceptible of two explanations:

A. Either that Type I bacilli are, on the whole, less virulent, or less toxogenic, in character than are those of Types II and III;

B. Or, that the serum prophylaxis in use until recently, afforded more adequate protection against Type I infections than it did against infections due to Types II or III.

As all those cultures of *B. tetani* that I obtained from serum laboratories conformed to Type I on serological examination, it appeared that hypothesis B was worthy of serious consideration.

II. Section IV deals with experiments that were undertaken to demonstrate *in vitro* the stability of the Types after prolonged culture, and to investigate the presence of antibodies other than agglutinins in the sera of animals immunised by inoculating

(*a*) the filtered products of growth—"Toxin,"

(*b*) washed bacilli,

(*c*) whole cultures.

The facts obtained from this section of the inquiry are:

1. That even when agglutinating sera of high titre are used the bacillary Types react specifically.

2. That even after very frequent sub-culture extending over a period exceeding one year, the bacilli remain true to type.

3. Anti-bacterial qualities, other than agglutinins, can be evoked by inoculating whole cultures of *B. tetani* into animals. For technical reasons the anti-bacterial body which was most fully studied was of the nature of a "stimulin" or "opsonin."

4. Antitoxic serum obtained by inoculation of culture filtrates does not stimulate phagocytosis of whole culture.

5. Inoculation of *washed* cultures does not evoke the elaboration of so active an "opsonin" (for *whole* cultures) as does inoculation of *whole* cultures.

6. *Washed* culture and *whole* culture appear to be equally active in evoking agglutinin production.

The following deductions therefore seem permissible: that in *whole* cultures of *B. tetani* three antigens, at least, appear to exist.

(*a*) The *bacillary* substance itself—the inoculation of this leads to the development of agglutinins which are specific to the Types.

(*b*) *An antigen*, which is anti-phagocytic, is present in young unfiltered cultures; but if present at all, is only found in small quantity in filtrates. The presence of this antigen in an *inoculum* evokes the development of "opsonins" which are specific to the Types.

(*c*) The *spasm-producing toxin*—an antigen which is filtrable and which, in laboratory animals at least, does not appear to be specific to the Types.

These findings suggest, that improvement might be looked for in serum prophylaxis and serum therapeutics from the employment of sera possessing anti-bacterial as well as antitoxic properties.

The demonstration of specific opsonic activity further suggests, that the typing of the bacilli may be of some import in the pathology of the disease.

III. In Section V the following subjects are considered:

(i) The mechanism of infection in tetanus.

(ii) The influence of antitoxic serum on infection with *B. tetani*, as contrasted with its influence upon intoxication with the products of that organism.

(iii) The relative value of *antitoxic* and *anti-bacterial* sera for prophylactic use.

(iv) The demonstration of "*whole animal*" immunity specific to the Types. This is the natural corollary of the experiments undertaken *in vitro* for the demonstration of *anti-bacterial* bodies specific to the Types. From the results obtained the following conclusions may be drawn:

1. That the *spasm-producing* toxin of *B. tetani*, when employed in sub-lethal doses, does not produce sufficient local devitalisation of tissue to permit of the growth of *B. tetani* when inoculated along with it.

2. That the toxin of *B. Welchii*, and to a less extent that of *Vibrion septique*, when used in sub-lethal doses, do produce sufficient devitalisation of tissue to allow of the development of tetanus infection. Antitoxins to the products of these organisms protect animals against infection with *B. tetani* when such products are used as tissue debilitants.

3. The protection afforded by tetanus antitoxin can only be partial, for, if the degree of tissue devitalisation be great, antitoxin used prophylactically fails to prevent the occurrence of tetanus.

4. The nature of the substance used for producing devitalisation of tissue exerts a profound influence upon the development of tetanus spores in the tissues. In guinea-pigs, saponin produces a lesion which always results in the development of spores of *B. tetani* inoculated along with it. The same reagent fails to initiate tetanus infection in the mouse. Trimethylamine, although it may cause the production of a large slough in mice, only infrequently causes the development of spores of *B. tetani* inoculated along with it. Calcium chloride of such concentration that it produces no obvious local lesion will almost invariably cause tetanus spores to develop in these animals.

5. Just as the products of *B. Welchii* and *Vibrion septique* induce tetanus *infection*, so certain other relationships, the nature of which is at present not determined, appear to depress the infectivity, or toxogenicity, of certain strains of *B. tetani*.

6. The experiments described in this section suggest, but do not prove, that while *monovalent antitoxic* sera exert no specific neutralising influence on the *spasm-producing* toxins of any one Type, they may nevertheless exert a specific *anti-infective* influence. This *anti-infective* influence is *quantitative*, but not *qualitative* in character.

7. The results so far obtained in the investigation of the relative value of *antitoxic* and *anti-bacterial* sera are equivocal. They suggest, however, that this is worthy of more extended investigation. This cannot be done until larger quantities of specific sera are available.

IV. In Section VI are discussed the results obtained in investigating the influence which various dressings exert upon the anaërobic flora of wounds. From this inquiry it is seen, that the dressings, which we were in a position to investigate, exert but little influence upon the anaërobes present in the wounds.

One important fact emerged from the investigation, namely, that excision of the wound area, irrespective of the dressings employed in treatment, exerts a beneficent influence. Anaërobe infection is relatively less when this procedure is employed and convalescence is established at an earlier date than when other methods have been used.

In connection with this work, experiments were undertaken to determine the antiseptic value of certain of the aniline dyes. It is found that the antiseptic activity of these may be greatly reduced in presence of fresh tissue, although their activity may not be reduced in presence of serum.

Tetanus bacilli may be found in wounds at any time during the process of healing. In one instance *B. tetani* was recovered from a wound 882 days after the infliction of the injury. It is sometimes difficult to demonstrate the presence of *B. tetani* in such circumstances, and several swabs may have to be taken before their presence is appreciated by the observer.

V. Examination of the blood of patients suffering from tetanus showed that agglutination could not be employed as an aid to the diagnosis of the disease—Section VII.

VI. In Section VIII is discussed the possible relationship which might exist between the serological Type of a tetanus bacillus, and the haemagglutinating Type of the individual from whom it was isolated. No relationship could be shown to exist between the two.

VII. In Section IX are discussed two interesting cases of abdominal tetanus.

VIII. In Section X are discussed the results obtained in making an examination of human faeces with a view to demonstrating the prevalence of the various Types of *B. tetani* in the human intestine. Unfortunately, owing to the numerous other and more pressing problems under investigation, this work could not be pursued with the vigour necessary to obtain adequate information which would permit of definite conclusions being drawn.

In conclusion we wish to record our thanks to our friends and colleagues who have assisted in the prosecution of this work by their advice and kindly criticism. To Major-General Sir David Bruce, K.C.B., F.R.S., A.M.S., we are especially indebted for inviting us to undertake the investigations, to Lt.-Colonel M. H. Gordon, C.M.G., R.A.M.C., for freely offered advice and encouragement.

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prosecution of the work. In no less degree do we appreciate the assistance of the surgeons and sisters in the various wards of these Institutions.

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Pte H. C. Wilson, R.A.M.C., by his untiring devotion to the work and by his help in many directions, has really made possible the successful prosecution of the investigation. We desire to record our appreciation of his assistance and warmly to appraise his energy and resource.