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REPORT

OF THE

ANTI-TYPHOID COMMITTEE.

1912.

APPOINTED ON 4800/6/1433A.

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FINAL REPORT ON 24/FOREIGN STATIONS/971.

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REPORT OF THE ANTI-TYPHOID COMMITTEE.

PART I.

CHAPTER I.

Introductory.

The Committee were appointed on the 16th March, 1904, by the Army Council and held their first meeting on the 11th May of that year at the Headquarters of the Army Medical Service, 68, Victoria Street.

The object of the Committee, as stated in the letters of invitation to the Members, was "to investigate the practical prophylactic and therapeutic value of current methods of immunisation against Enteric Fever."

Surgeon-General Sir Alfred Keogh, K.C.B., the Director-General of the Army Medical Service, took the chair at the first meeting and further explained the objects of the Committee and the proposed agency for the carrying out of the contemplated investigations.

Personnel of the Committee.

Colonel David Bruce, F.R.S., Army Medical Service.
William Bulloch, Esq., M.D., London Hospital.
F. Foord Caiger, Esq., M.D., F.R.C.P., Medical Superintendent, South-Western Fever Hospital.
James Galloway, Esq., M.D., F.R.C.P., Charing Cross Hospital, Member of the Advisory Board of the Army Medical Service.
Major W. B. Leishman, R.A.M.C., Professor of Pathology in the Royal Army Medical College.
Robert Bruce Low, Esq., M.D., Local Government Board.
Professor A. Macfadyen, Lister Institute of Preventive Medicine.
C. J. Martin, Esq., M.D., F.R.S., Director of the Lister Institute of Preventive Medicine.
A. E. Wright, Esq., M.D., F.R.S., Lecturer on Bacteriology, St. Mary's Hospital.

Chairman.

The Committee, at their first Meeting, unanimously appointed Dr. C. J. Martin, F.R.S., as their Chairman, and he has served in that capacity throughout its existence.

Changes in the Constitution of the Committee.

1. Subsequent to the 8th Meeting of the Committee, held on October 12th, 1904, the following Members resigned :—

William Bulloch, Esq., M.D.
A. E. Wright, Esq., M.D., F.R.S.

2. To the great regret of the Committee they lost the valuable services of Dr. Allen Macfadyen through his death on 1st March, 1907.

3. J. Rose Bradford, Esq., M.D., F.R.S., was appointed to the Committee on 8th March, 1907.

4. George Dean, Esq., M.D., was appointed to the Committee in place of the late Dr. Macfadyen on 11th March, 1907.

Present Constitution of the Committee.

Chairman.

Dr. C. J. Martin, M.D., F.R.S.

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Members.

Sir JOHN ROSE BRADFORD, M.D., K.C.M.G., F.R.S.
Colonel Sir DAVID BRUCE, C.B., F.R.S.
Dr. F. FOORD CAIGER, M.D., F.R.C.P.
Dr. G. DEAN, M.D.
Dr JAMES GALLOWAY, M.D., F.R.C.P.
Lieut.-Colonel Sir WILLIAM LEISHMAN, R.A.M.C., F.R.S.
Dr. BRUCE LOW, M.D.

Secretary.

This duty was performed by Lieut.-Colonel Bruce Skinner, M.V.O., R.A.M.C., for the major part of the period covered by the work of the Committee and, after his departure from the War Office for India, Lieut.-Colonel Melville, R.A.M.C., and Mr. E. T. Gann, have, in succession, filled the post.

The Agency by which the experimental work detailed in the Report was carried out.

After full discussion at the earlier meetings of the Committee it was agreed that this work should be entrusted to one of their number—Major Leishman, the Professor of Pathology at the Royal Army Medical College—and to such other officers of his corps as he required to collaborate with him. Sanction was obtained from the authorities for the attachment of such officers to the Pathological Laboratory of the Royal Army Medical College for various periods and the whole of the work shortly to be detailed has accordingly been done by these officers. Throughout the investigations Major Leishman has been in touch with the Committee on the nature and results of this work; presenting schemes for the future for the approval of the Committee and submitting, from time to time, detailed reports of that which had been done.

Since the commencement of the work in 1905, the following officers have collaborated with Major Leishman for varying periods:—

Major W. S. Harrison, R.A.M.C.
„ H. W. Grattan, R.A.M.C.
„ S. L. Cummins, R.A.M.C.
Captain A. L. A. Webb, R.A.M.C.
„ A. B. Smallman, R.A.M.C.
„ C. C. Cumming, R.A.M.C.
Lieutenant F. M. G. Tulloch, R.A.M.C.
„ R. G. Archibald, R.A.M.C.

The greater part of the experimental work, being of scientific interest, has been published from time to time in medical and scientific journals, of which the following is a list:—

1. Journal of the Royal Army Medical Corps, Vol. V., page 1, 1905.
2. „ „ „ „ Vol. VIII., page 472, 1907.
3. „ „ „ „ Vol. X., page 583, 1908.
4. „ „ „ „ Vol. XI., page 327, 1908.
5. Journal of the Royal Institute of Public Health, Vol. XVIII., July, August, and September, 1910.

The above publications gave full particulars of the details of the various experiments, and it is not thought necessary in this Report to repeat the whole of them, but those which resulted in definite conclusions have now been collated and are given in the Report, freed as far as possible from technical detail, and unaccompanied by the protocols which appeared in the earlier publications. It has, however, been felt well to reprint some of the charts in connection with the experimental work for the sake of more clearly illustrating the text.

The Committee have delayed the publication of the Report until the present day for the following reason. As will be seen, they felt it necessary to await the accumulation of sufficient statistical evidence of the effects of the vaccine, collected in accordance with a definite system which they had drawn up. This material was to be obtained from regiments sent on foreign service from this country and, naturally, it has taken some years to allow of the accumulation of figures sufficient to enable them to frame definite conclusions as to the effect of the modifications of the vaccine resulting from the experimental work.

Sufficient material having now accumulated to allow of such judgments being formed, the Committee feel that their Report should no longer be delayed; but, at the same time, they realize that progress must be continuous, and they trust that the researches on typhoid vaccine will be continued, with a view to the introduction of still further improvements in the system in the light of recent progress in science.

CHAPTER II.

Short History of Typhoid Inoculation.

The fact that an attack of enteric fever confers as a rule an immunity against a subsequent attack has been held as proven for long and, ever since the discovery of the Bacillus typhosus by Eberth in 1880, and its isolation in pure culture by Gaffky in 1884, bacteriologists have attempted to reproduce this immunity in laboratory animals by various measures. A fair amount of success attended these earlier methods of immunisation but the results were not thought sufficiently striking to allow of the application of any of them to man.

The next important step in advance came with the discovery by Pfeiffer and by Kolle of new substances in the blood of immunised animals which were capable of analysis and measurement and which permitted an estimation being made of the degree of immunity which had been reached by any given method of immunisation. The history of the discovery and investigation of these substances forms a large part of the modern history of bacteriology and does not lend itself to useful summarising, so all that need be said here is that the chief of these substances were the agglutinins, the bactericidal substances, the bacteriolysins and, more recently, the opsonins. Some or all of these substances were found to increase largely in amount as a result of the various immunising procedures and were found to bear a direct relationship to the degree of immunity conferred.

Investigation soon showed that similar substances were present in the blood of cases of enteric fever and that they persisted in the blood for many months after convalescence. From these observations it was concluded that the immunity conferred either by an attack of enteric fever or by some process of artificial immunisation in the case of an animal were attributable to the presence of these substances in the blood and fresh encouragement was thus given to the hopes that by applying one of these processes of artificial immunisation to man, a useful degree of protection might be induced against an attack of the disease.

The first attempts in this direction were made independently, by Sir A. E. Wright[1] and by Pfeiffer and Kolle,[2] both of whom inoculated dead cultures of typhoid bacteria into man with no bad effects and with the result that specific agglutinins and bactericidins made their appearance in the blood of the inoculated men. All of these workers, however, have confessed their indebtedness to the earlier work of M. Haffkine, whose experiments upon cholera vaccination in man had already proved that the inoculation of dead cultures of Koch's vibrios produced the appearance of specific substances in the serum of the inoculated and had also been able to show that this was accompanied by an actual protection of the individual against an attack of Asiatic cholera.

In the years following these first attempts at active immunisation of man against enteric fever, the progress which was made in connection with the subject is unquestionably due to Sir A. E. Wright, who, in addition to his unremitting researches in the laboratory, carried out a large number of inoculations with the vaccine which had been devised by him. An account of this earlier work of himself and his colleagues at the Army Medical School at Netley has been published by him in book form,[3] and need not be recapitulated here, but it should be borne in mind that it is largely owing to the untiring labours of Sir A. E. Wright in those early years that typhoid inoculation has come to be recognized as a practical means of protection against the disease.

It was not until the publication of the statistical results of these first inoculations that the method was adopted on any scale in other countries, but the vaccines used in

[1] "Lancet." 19th September, 1896.
[2] "Deutsche Medicinische Wochenschrift." 12th November, 1896.
[3] "A Short Treatise on Anti-Typhoid Inoculation." A. Constable & Co., 1904.

such instances have mostly been prepared by different methods and, with few exceptions, the numbers have been too small to afford any criterion of their comparative value, while many suggested vaccines have not been tested at all outside the laboratory. The best known of these vaccines are those which were devised by Levy in Austria, by Pfeiffer and Marx, by Shiga, by Brieger and Mayer, by Bassenge and Rimpau, and by Wassermann in Germany, by Besredka in France, and by Dzeergowsky in Russia.

The only tests of any statistical importance, other than those afforded by Sir A. E. Wright's vaccine, have been derived from the use of Pfeiffer and Kolle's vaccine on some of the German troops engaged in the Herero rebellion in German South-West Africa in 1904. The published results in this instance were favourable to the protective power of the vaccine, but appeared inferior to those obtained by Wright's vaccine. In the case of the latter, the earliest records were in connection with epidemics of enteric fever which occurred in the British Isles, notably at Maidstone in 1897, and at the Richmond Asylum in Dublin in 1900. Sir A. E. Wright had also the opportunity of testing his method on some of the British troops serving in India, to which country he was called in 1899 to serve upon the Indian Plague Commission. In several instances he was successful in obtaining a large number of the soldiers of a regiment to volunteer for inoculation, and in some of these cases the subsequent incidence of enteric fever afforded a valuable indication of the benefits of the process.

At the end of 1899 the outbreak of the Boer War offered a chance of an extended trial and Sir A. E. Wright obtained the sanction of the War Office authorities to carry out his system of inoculation on such men as should voluntarily present themselves prior to their embarkation for the seat of war. The men availed themselves of this on a large scale, and, according to Lieut.-Colonel R. J. S. Simpson's recent account of the Medical History of the Campaign,[1] there are official records of the inoculation of 14,626 men out of a total strength of 328,244 men who served during the 3 years of the war. Owing to the difficulties of obtaining accurate statistical information, which appear to be inseparable from active service in the field, the evidence in the case of typhoid vaccine and its power to protect against attack proved disappointing and it was only in certain units that the material was of sufficient homogeniety to be of value. All the material which had been accumulated up to 1904 was placed before the Committee and most carefully studied by them at their first meeting, and their report upon these figures will be found recorded in the minutes of their meeting held on 17th June, 1904, and were incorporated in the Interim Report made by them on the 8th July, 1904.

As a result of their enquiry into the whole of the available statistical evidence the Committee recommended to the Army Council in their Interim Report, *q.v.*, that the system of inoculation, which had temporarily been in abeyance, should be re-introduced into the Army as a voluntary measure, but, in addition to this, they recommended that investigations should be commenced with a view to the introduction of possible improvements in the system introduced by Sir A. E. Wright. Acting on the recommendation of the Committee sanction was given by the Army Council for the recommencement of the inoculations and also for the carrying out of the experimental work which the Committee had in contemplation. The work in question now falls to be described.

The first portion of the experimental work which the Committee contemplated was the systematic investigation of the blood changes which follow the inoculation of typhoid vaccine, and, as soon as the re-adoption of the system had been sanctioned, this was proceeded with, the experiments being carried out by Lieut.-Colonel Leishman and his colleagues, Major Harrison and Lieutenants Smallman and Tulloch. An account of this work will first be given and this will be followed by a description of the rest of the experimental work. Part II. of the Report deals with the statistical results which have been collected during the existence of the Committee.

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[1] Journal of the Royal Army Medical Corps, Vol. XV., page 414, 1910.

CHAPTER III.

Investigation of the Blood-changes which follow Typhoid Inoculation.

As the result of the work of Sir A. E. Wright and others, many of these changes were known at the time the investigation was commenced, for instance, the appearance of the specific agglutinins and the rise in the bactericidal power of the blood serum; but it was felt that useful information might result by the systematic quantitative measurement of the various immune substances for a period of some weeks following inoculation, in other words, by the building up of "immunity curves" of the various substances in question. In this way it was hoped that useful light would be thrown on such points as the most appropriate dosage, the proper interspacing of the doses, the possible existence of a negative phase, &c.

As soon, therefore, as sanction had been obtained, steps were taken to organize such an investigation of the blood of a number of men inoculated with typhoid vaccine. Choice was made of the 2nd Bn. Royal Fusiliers, at that time stationed at Aldershot, as being the only unit of any size which was proceeding to India during the trooping season at that time in progress. Every assistance was provided by the Commanding Officer of the regiment, and by the medical authorities at Aldershot, while advantage was taken of the presence of a small laboratory belonging to the District Sanitary Officer, for the carrying out of the analytical work.

Although but a small proportion of the men who were to proceed abroad were present at the station at the time of the commencement of the work, many being absent on furlough pending embarkation, those who were present at a lecture given to the men by Major Leishman responded well, about 50 per cent. coming forward as volunteers for inoculation. A special appeal was also made for a small number of those inoculated to attend daily for the purpose of having samples of their blood taken with which to carry out the tests detailed below. These men were secured without difficulty through the assistance of the regimental authorities, on being excused morning parade during the course of the experiment.

General purpose of the experiment.

The following were the principal points to which attention was directed:—

1. The construction of "immunity curves" of the substances which appeared in the blood in response to the inoculation of the vaccine. Such curves were built up from the results of the daily quantitative tests carried out on the blood of the inoculated.

2. A comparison of the effects of different doses of the vaccine as regards the symptoms following inoculation and the development of the protective substances. From such a comparison of the results obtained in connection with the groups of men receiving different doses, it was hoped that light would be thrown upon the optimum dose of the particular vaccine employed.

3. The determination of the effect of two consecutive inoculations, and of the most appropriate moment for giving the second dose.

4. Investigations as to whether any evidence was to be gained from the daily analyses of the protective substances with regard to the production of a "negative phase" of resistance as a result of inoculation, the duration of such a phase, if it was found to occur, and its relationship to the dosage of the vaccine.

The vaccine employed.

Full details as to the preparation and the method of standardisation employed are to be found in the detailed report already published,[1] and it will suffice here to state that, in accord with Sir A. E. Wright's recommendations, it consisted of a young broth culture of a strain of B. typhosus which was of very low virulence. The broth culture was sterilised by heating at an average temperature of 62° C. for a quarter of an hour. (The temperature employed on this occasion should be borne in mind, as it has an important bearing upon some of the later experimental work, and because it was probably accountable for the poor results, as regards protection, which were subsequently obtained in this regiment, *see* page 58). After proof of sterility, by both aerobic

[1] Journal of the Royal Army Medical Corps, Vol. V., page 1, 1905.

an d anaerobic cultures, a small quantity of lysol, 0·5 per cent., was added as a safe gu ard against subsequent contamination.

As regards standardisation, this was attempted by the enumeration of the numbe of germs by means of the mixture of a measured quantity of the vaccine with a know dilution of normal blood, and a comparison of the relative numbers of the germs an of the red blood cells, as described by Sir A. E. Wright. The results, however, wer not altogether satisfactory, and further help in the estimation of the bacterial content of the vaccine was obtained from the chairman, Dr. C. J. Martin, who was good enoug to estimate the weight of the dried bacterial bodies in a measured quantity of th vaccine and, by means of a series of control experiments with emulsions of typhoi germs, to obtain a correlation between the weight of such a bacterial residue and th number of germs contained in a given volume of that vaccine. From the combine results of these experiments the conclusion was reached that the vaccine employed i the inoculations was of a strength equivalent to 1,700 million dead bacteria per c.cm A considerable amount of experimental work, which was subsequently undertaken wit a view to possible improvements on the system of standardisation, showed that thi result can only be regarded as approximately accurate.

Dosage.

It was decided to inoculate the general body of the volunteers with one-third of a c.cm. for the first, and two-thirds of a c.cm. for the second dose, these amounts corresponding, respectively, to 566 millions and 1,133 millions of bacteria. These doses were decided on in the light of earlier experiences of inoculation and of the local and general effects which had followed the preliminary inoculation of the officers conducting the investigations.

From this general body of the volunteers six men were chosen for the purpose of carrying out on their blood the daily tests for the various protective substances; these constituted Group "B."

In order to test the effects of doses of vaccine, higher and lower than this, two other groups of men were selected: the first of these, Group "A," received double the amount given the men of Group "B," at both first and second inoculations; while the second group, "C," received but one-third of each of the two doses given to the general body of the men from whom, as has been said, Group "B" was formed.

In addition to these three groups, "A," "B," and "C," a fourth was constituted as the opportunity was found to offer of testing a point of some scientific interest in connection with protective inoculation. This was the possible power of individuals who had been inoculated some time before to respond to the re-inoculation of a very small dose of vaccine by an unusually large output of protective substances into the blood. In the case of this regiment it was found, before the experiment started, that a certain number of men had been inoculated, 5 years before, on proceeding to the South African campaign. Five of these men were persuaded to be re-inoculated and they thus constituted Group "D" and received as a first dose $\frac{1}{30}$th of the amount of vaccine given to the general body. As will be seen, the response to this small dose was so slight that it was considered advisable to increase the second dose disproportionately, and they were given accordingly 10 times their initial dose at their second inoculations.

The details of the various groups, and of the reactions following the inoculation of the doses respectively given to them, need not be repeated here. It will suffice to state in general terms that the reactions, both general and local, were of the usual type and quite moderate in character, even in the case of the group which received the largest quantity of vaccine. In no case did the symptoms extend beyond the second day.

The measurement of the protective substances.

It was proposed to estimate daily the amount of the following substances present in the blood of each of the four groups:—

1. Agglutinins.
2. Bactericidal substances.
3. Bacteriolysins.
4. Opsonins.
5. Stimulins.

For this purpose it was decided, as had been suggested by Sir A. E. Wright, to "pool" the samples of blood collected daily from the men of each group, and to carry

out the tests on the mixture of their blood serums thus obtained. It was thought that it would be possible in this way to obtain a better idea of the average development of the various substances than by relying upon the results obtained in the case of one individual. Considerable personal variations were already known to exist in respect of the development of these substances, and it was therefore thought that the above system might eliminate this fallacy to a great extent. Previous work in connection with this method of pooling had been done by Major Leishman and his colleagues before the inoculations were undertaken, and the results showed that the system *did* permit a fairly accurate measurement of the average values of the various protective substances which develop in the blood of a group of men.[1]

The results of the tests may now be considered.

1. *Agglutinins.*—In all four groups the first appearance of a rise of these substances, above their normal level, occurred upon the 9th day after the first inoculation. Dosage, therefore, would appear to have little to say to the hastening or retarding of their appearance. Once they made their appearance there followed a very rapid rise, and the heights ultimately attained were very considerable in the case of Groups "A," "B," and "C," and marked also, considering the very small doses, in the case of Group "D." Following the rapid initial rise, a fall occurred in all cases from 4 to 5 days after reinoculation; and subsequently to this there was a second rise to a level higher than that previously attained, presumably as the result of the second dose of vaccine, since the same interval of 9 days was found to elapse between this secondary rise and the second dose of vaccine, as had been noted in connection with the first dose.

As regards the comparative heights reached in the different groups, it was found that the initial rise was greatest in the case of "C," and lowest in the case of "A" (excluding "D," in which the figures were low throughout), while this order was reversed after the third week, the height of the agglutinins at the end of the experiment standing in direct proportion to the size of the doses given to each group. (*See* Chart I.)

2. *Bactericidal substances.*—A considerable amount of preliminary work was done by Major Leishman and his colleagues in order to select the best method of measuring the bactericidal action of the blood serum of the inoculated men. Sir A. E. Wright's method was adopted, with certain modifications which appeared called for as the result of some of these experiments. The blood drawn from the finger of each man of the particular group concerned was allowed to clot in the sealed capsules used for this purpose and, at the end of 2 hours, the serum was drawn off from the clot and equal volumes of each man's serum were mixed together to furnish the "pooled" serum with which the tests were carried out. Every possible care was taken to ensure that the conditions under which this was done were as uniform as posible, in order that the results of one day's observations might be fairly comparable with those of the next.

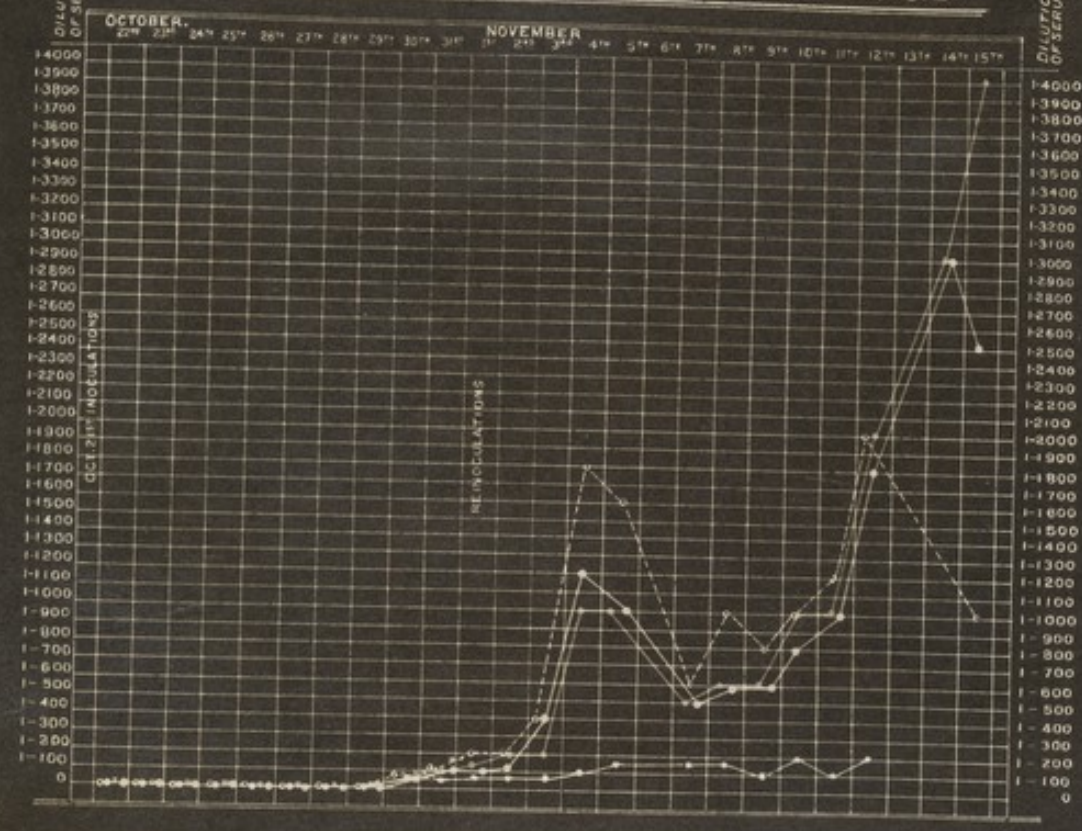
The general principle of the test was the ascertaining of the extent to which the serum of each group could be diluted with an inert fluid before it lost its power of killing off a certain definite number of typhoid bacteria. For this it was necessary to prepare a series of dilutions of each pooled serum, and to mix each of these dilutions with the same "test dose" of typhoid bacteria. The test dose adopted was a given volume of a 24-hours' broth culture of typhoid which had been diluted 10,000 times. Previous experiment had shown that the average number of bacteria present in this test-dose was from 200 to 300, but a control experiment was made daily to ascertain the actual number present in each day's tests. The task now imposed upon each dilution of the pooled serum was the killing of these 200 or 300 germs within one hour, at blood heat. To ascertain whether this had been accomplished, each tube's contents were blown out on to agar plates which were incubated for 20 hours, at the end of which time the results were noted and recorded. When the serum had been strong enough to kill all the germs the plates naturally remained sterile, while, if the bactericidal substances had been too highly diluted, evidence of this was found in the increasing number of colonies which developed on the plates.

In fixing the "end-point" from which to frame the records and curves, it was decided, for reasons which were fully explained,[2] to take as evidence of a negative bactericidal effect the lowest dilution of the serum in which it was found that two or

[1] Journal of the Royal Army Medical Corps, Vol. V., page 20, 1905.
[2] *Idem*, page 21, 1905.

CHART I.

COMBINED ACCLUTINATION CURVES OF GROUPS A.B.C.& D.



more bacteria had survived. The dilution next below this, *i.e.*, the one which contained the serum in stronger concentration, was adopted as the end-point and the standard of measurement of the bactericidal power.

The method was one of considerable technical difficulty but on the whole it yielded results which were regular and easy of interpretation.

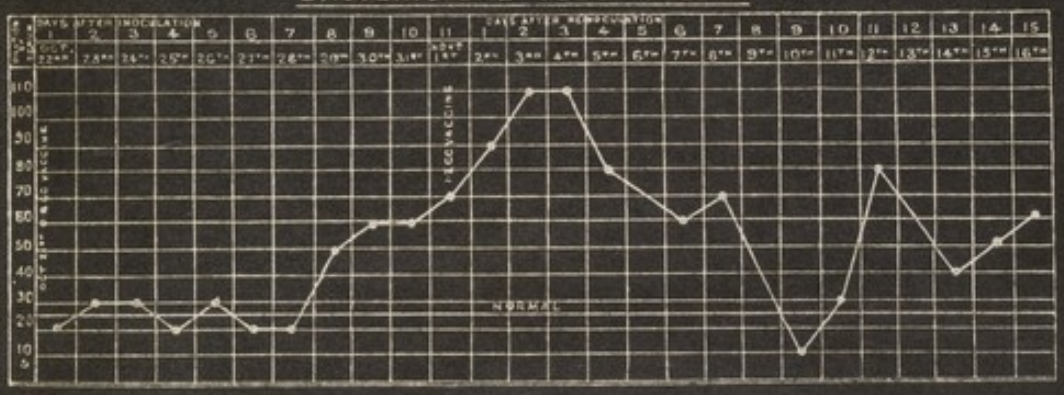
In drawing up the curves to illustrate the rise and fall of these bactericidal substances in the blood of the inoculated a normal base line was adopted at the level of serum diluted 1– 25. This value had been fixed on by preliminary experiments with the method upon the blood of healthy young men who had not been inoculated and had not, at any time, suffered from enteric fever. Individual differences were naturally encountered in these experiments and the figure mentioned was the average of all the observations on these normal men, while, as has already been stated, the system of pooling appeared to minimise any fallacy due to these individual differences.

Turning now to the results of these observations of the bactericidal power, no change outside the limits of normal variation was found to occur for the first 6 days following on the inoculation of the various groups. The first noticeable rise above the normal level occurred on the 7th day in the case of the "B" and "D" groups and this was followed by similar rises in Group "A" on the 8th day and Group "C" on the 9th. During the succeeding days the levels attained rose more or less continuously and, in the main, in proportion to the dose of vaccine administered. It is to be noted that there was no evidence of any negative phase reflected in the earliest estimations of the bactericidal power, even in the case of the group which received the largest dose. (*See* Charts II. and III.)

CHART II.

GROUP "A."

"BACTERICIDAL SUBSTANCES."



GROUP "B."

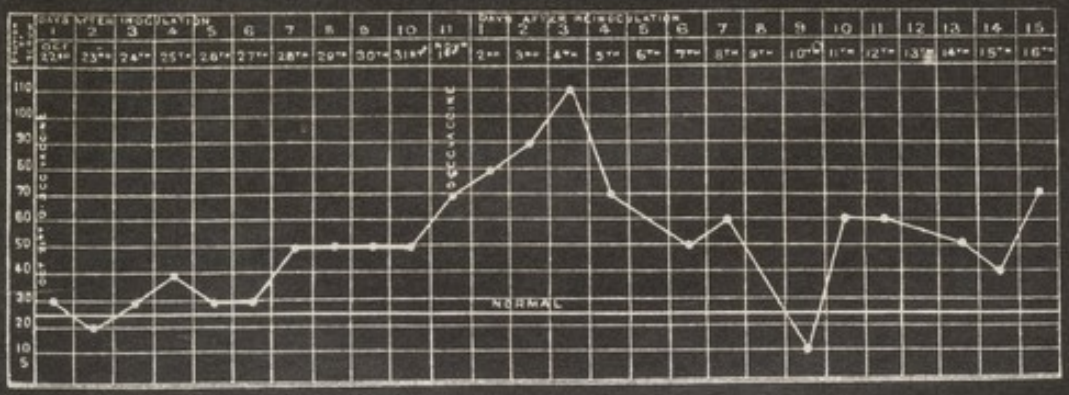
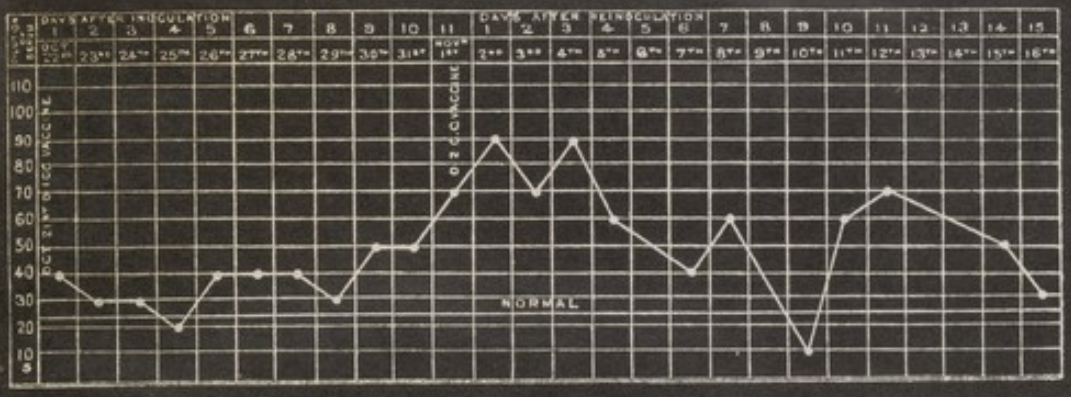


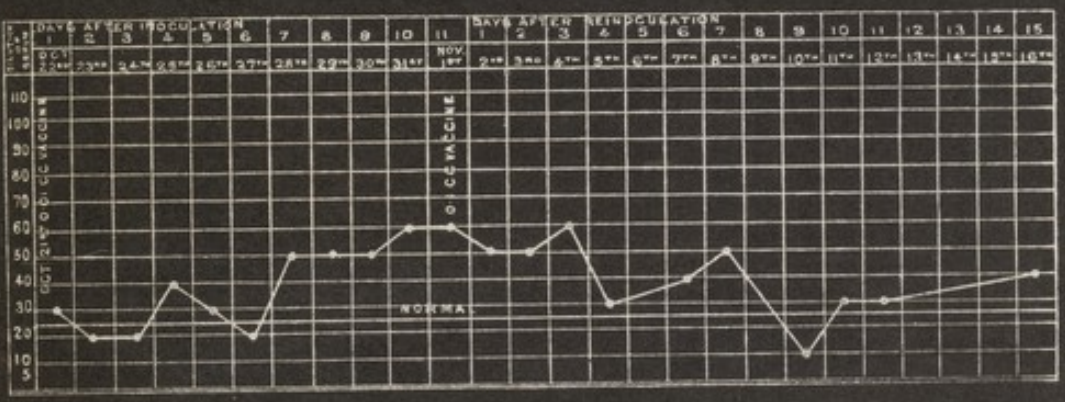
CHART III.

GROUP "C."

"BACTERICIDAL SUBSTANCES."



GROUP "D."



At the time of the administration of the second doses the bactericidal power of the various groups appeared still to be rising and, during the days immediately following these second doses, it rose still higher and reached the points of maximum value in the case of Groups "A," "B," and "C." In Group "D" there had been very doubtful evidence of any increase as a result of the first small dose of vaccine while the second inoculation with a ten-fold larger but still small dose also failed to show any increase in the amount of the bactericidal substances.

In all cases the maxima were reached on or before the third day after reinoculation, subsequently a decline commenced which was interrupted on the 8th and 9th days by a fall to or below the normal level, which was attributed to an experimental error, but may, possibly, have been a true reading. At all events, it was rapidly recovered from, and, at the conclusion of the experiment, 15 days after the second inoculations, the bactericidal power of the two Groups "A" and "B," those, namely, which received the high and medium dose, respectively, were still considerably above the normal limits of variation. On the other hand, the value of Groups "C" and "D" had, at that time, fallen within these limits.

3. *Bacteriolysins.*—Assuming that the substances which kill the bacteria, in other words the "bactericidal substances" just dealt with, are distinct from those which are responsible for their disintegration after death, an attempt was made to measure the development of the bacteriolytic substances in the blood of the inoculated, and to this end, before proceeding to Aldershot, a method was devised for the estimation of these bacteriolysins. It was felt at the time that the method adopted was lacking in precision, but it served at least to demonstrate the fact that the bacteriolytic powers of the blood

were markedly increased as a result of the inoculations, and that the degree of this power corresponded directly with the dose of vaccine given; there was further noted a fairly close accord with the values attaching to the bactericidal power of the groups on the same days.

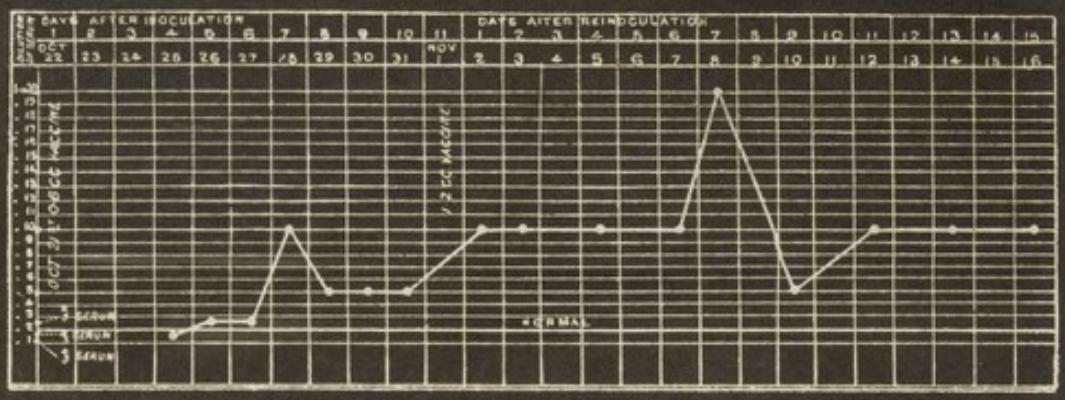
It is now considered doubtful whether any distinction can be drawn between the bactericidal substances and bacteriolysins, and the tendency at the present moment is to regard them more as different manifestations of the action of one substance or group of substances.

In the method employed a series of dilutions of the serum of each group was prepared, and each of these dilutions was mixed with an equal volume of a 24-hours' broth culture of typhoid bacilli, undiluted except for this addition of the serum; the mixed serum and bacteria were then left in contact at blood heat for one hour. They were next blown out on to slides and the resulting films were stained and examined microscopically for the evidences of bacteriolytic action, as manifested by the alteration or complete disappearance of the bacteria. Various arbitrary standards were adopted, and, from the readings thus obtained, charts were drawn up for each group of the inoculated; the normal line, as in the case of the bactericidal substances, being fixed at the level determined from a series of similar observations upon the blood of normal men.

CHART IV.

GROUP "A."

"BACTERIOLYSINS"



GROUP "B."

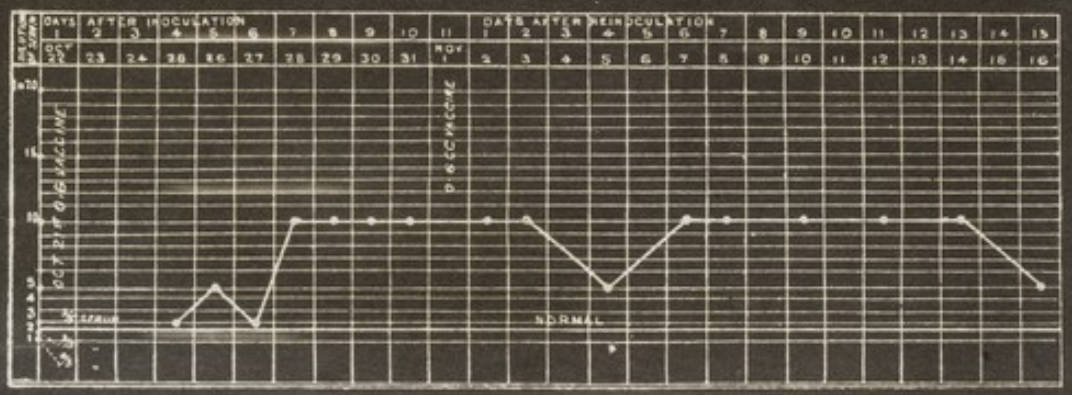
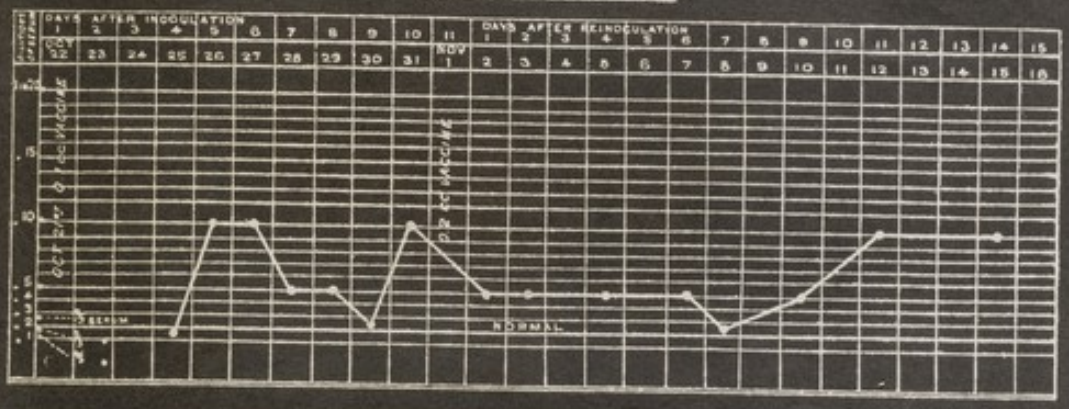


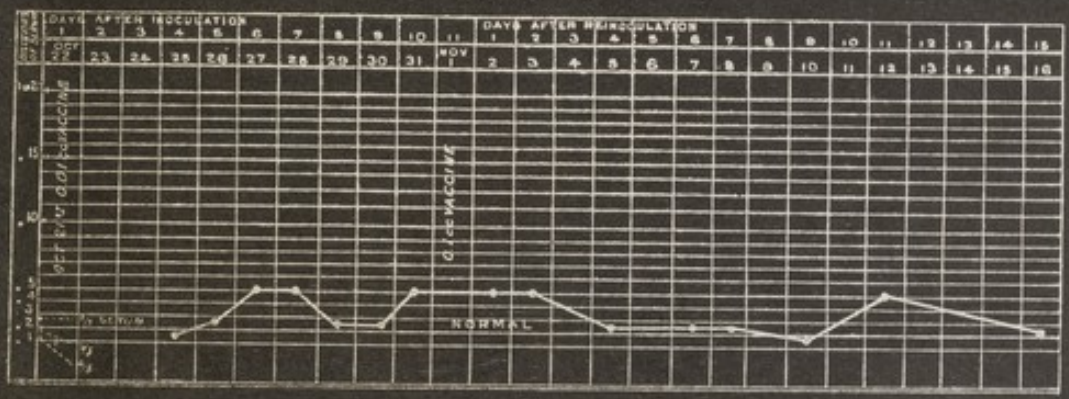
CHART V.

GROUP "C"

"BACTERIOLYSINS"



GROUP "D."



The results of the daily tests showed that the bacteriolytic powers of the blood were markedly raised by the inoculations, but this was only manifest in the case of Groups "A," "B," and "C," the highest levels being recorded in Group "A," that which received the largest doses of vaccine. Group "D," on the other hand, failed to show any definite evidence of an increase of these substances on account, probably, of the very small quantities of vaccine used on this group. No evidence of any negative phase was forthcoming from this series of observations. (*See* Charts IV. and V.)

4. *Opsonins.*—An attempt was also made to obtain a curve of the development of these substances, but at the time of the experiment comparatively little was known of the nature and properties of typhoid opsonins, and the technique which was devised for the purpose did not yield very satisfactory results. The chief reason for the comparative failure of the tests in question was only made clear when further experience showed that the typhoid opsonin which develops during immunisation differs from the typhoid opsonin present in normal serum in being thermostable, the latter being thermolabile and readily destroyed by heating the serum to 60° C. In the technique in question it was assumed that Sir A. E. Wright was correct in considering that typhoid opsonins were thermolabile, and the thermostability of the immune opsonins only became manifest as the experiment progressed, and too late to allow of the adoption of another system of measurement. Under these circumstances, therefore, it is thought unnecessary to reproduce here the account of the opsonic estimations which was given in detail in the account already published.[1]

As will be seen later, once the thermostability of the immune typhoid opsonin was recognized and a suitable method of measuring these adopted, much valuable information was obtained as to the progress of immunisation in man and in experimental animals.

5. *Stimulins.*—A fifth series of investigations was also undertaken with a view to estimating substances which were supposed by Major Leishman and his colleagues to favour phagocytosis by directly stimulating the leucocytes, as opposed to the sensitising

[1] Journal of the Royal Army Medical Corps, Vol. V., page 25, 1905.

action of opsonins upon the bacteria. The name stimulin had been given to such theoretical substances by Metchnikoff at an earlier date, and it was assumed that the action noticed by Major Leishman, on adding a trace of an immune serum to a normal serum, was due to such a stimulin substance. Here, again, later experience has shown that the existence of these stimulins, as separate substances, requires further demonstration, in view of the thermostability of the immune typhoid opsonins. One of the chief reasons which led to search being made for them was that the stimulating action had been shown to be due to a thermostable substance, and therefore to something which was distinct from typhoid opsonins, the latter, as has been said, being thought at that time to be invariably thermolabile.

As regards the general results of the investigation the following conclusions were drawn:—

(1.) The quantity of vaccine given, even in the case of Group "A" which received the highest amount, had not resulted in local or general reactions of any undue severity, and the effects of inoculation had passed off, in almost all cases, within 48 hours. The severity of the reactions was noted to correspond to a certain extent with the amount of vaccine used. The relative severity of the first and second doses was inconsistent; it appeared however, that, in those who had the largest initial dose, the second inoculation of double this quantity, produced a smaller degree of discomfort than the first. On the other hand, those who had received but a small quantity of vaccine as a first inoculation suffered rather more at their second inoculation, when receiving twice what was given at the first dose.

(2.) The effects of the inoculations upon the blood was the development of a remarkable rise in the agglutinins, bacteriolysins and bactericidal substances. These rose to heights far above the normal range and, even in the case of the group which received the smallest doses, remained well above the normal at the termination of the month during which the observations lasted.

(3.) As regards the relationship between the dose of the vaccine and the subsequent development of the above substances, while the advantage appeared to lie with the Group "A," which received the largest dose, the values in this instance were but little above those recorded in Group "B," which received but half the quantity of vaccine given to "A." In Group "C," which received one-sixth of that given to "A," the values recorded were distinctly lower but, still, by no means in proportion to the difference in dosage.

Taking into consideration the reactions as well as the development of these protective substances the most suitable dose would appear to have been that given to Group "B," namely 566 million for the first inoculation and 1,133 million for the second. (It may be added here that, although the vaccine itself has been modified from time to time, in the light of the experiments detailed further on, the above-mentioned first and second doses of dead bacteria have been approximately adhered to and are still in use.)

(4.) The various tests showed no evidence of the occurrence of a negative phase of resistance as an immediate result of inoculations, with the doubtful exception of the transient fall in the bactericidal power eight or nine days after reinoculation. As the first tests for the various substances were instituted within 16 hours after the initial dose had been given it would appear that, if such a phase had resulted, it must have been of a very ephemeral nature. The system of pooling the blood of several individuals of the same group admits the possibility of such a phase having occurred in individual cases but, had this been common, or even well marked in a single instance, it could hardly have failed to manifest itself in the daily analyses. In deciding upon the adoption for general use of the dose given to Group "B," in preference to that given to Group "A," this question of the possible danger of a negative phase was borne in mind, as there can be little doubt that the larger the dose of the vaccine the greater the likelihood of such a temporary diminution of resistance to infection.

(5.) The interval of 10 days between the administration of the two doses, adopted in this experiment, appeared to be satisfactory; at that time the protective substances had already made their appearance, and the immediate effects of re-inoculation were not accompanied by any fall in the amount of these substances; on the contrary, it would seem to have stimulated their further elaboration.

(6.) The employment of a very small dose of vaccine in the case of the group of men "D," who had been inoculated 5 years before, did not confirm the anticipation that

they would exhibit an exceptional response to such a dose in elaborating large quantities of protective substances.

In order to trace the subsequent fluctuations of the protective substances, one of the officers engaged in the above work, Captain A. B. Smallman, accompanied the regiment to India, and was attached to it for 3 years.

The regiment arrived at Lebong, adjoining Darjeeling, on 9th January, 1905, but, for various reasons, it was found impossible to commence the various tests until 13th February. The first observations made on the groups, approximately 4 months after the first inoculations given at Aldershot, showed that the agglutinins were still high, and that they then stood in direct ratio to the dose of vaccine given; the values in the four Groups, "A," "B," "C," and "D," were, respectively, 1,000, 600, 200 and 50. The bactericidal power, on the other hand, had in most cases fallen within the limits of normal variation, as these had been determined by the preliminary work of Major Leishman and his colleagues. The subsequent analyses of these substances made by Captain Smallman gave very low and irregular readings, although the technique, with which he was thoroughly familiar, was rigidly adhered to and identical in every respect with that employed at Aldershot. It appeared probable, therefore, that some essential factor in the experiment had not remained constant. From his later experiments with the blood of the inoculated, and others which he devised with a view to controlling his technique, it would appear that this altered factor was to be found in the different conditions of growth of the culture of the typhoid bacillus which was used throughout all the experiments. At home no difficulty is experienced in procuring meat of good quality, from which to prepare the culture material used for growing the bacilli, but this is far from being the case in the tropics. It was found that the growth of the typhoid bacteria in the nutrient broth prepared in Darjeeling was greatly restricted, the number of bacteria which developed in 24 hours averaging only about one-third or one-fourth of what had been obtained at Aldershot. The conditions of growth, therefore, being obviously less suited to the requirements of the strain of typhoid bacteria it is probable that they were, at the same time, rendered more sensitive to the bactericidal power of blood serum, and that in consequence the readings obtained at Aldershot and Darjeeling, respectively, cannot be taken as strictly comparable. The above supposition appears supported from the results of the tests which were subsequently carried out on the blood of normal men, the readings found being distinctly lower than the average of the estimations obtained at home.

The bacteriolytic substances were also found to have fallen to the normal in the case of all of the groups at the time of the first Darjeeling observations, four months after the first inoculations. In these observations, also, it would appear that the irregular results at times in evidence might be attributable to the greater sensitivity of the bacteria growing under comparatively unfavourable conditions.

The estimations of the phagocytic powers of the blood, as tested by the observations made of the opsonin and the stimulin ratios, revealed similar irregularities, and the only definite point which appeared to emerge from them was the confirmation of the fact noted at Aldershot that persistently higher readings were found in the case of the serum which had been previously heated with a view to the destruction of the supposed thermolabile opsonins.

Similar tests were continued at intervals during the stay of the regiment at Darjeeling, and, subsequently, when the regiment moved from this station to Secunderabad in November, 1905. To summarise the results yielded by these further tests, it may be said that, in the case of all of the groups, no evidence of the presence of protective substances above their normal amounts could be found later than 6 to 9 months after the inoculations had been carried out. From this standard, then, it would appear that the inoculated individuals by this time had lost the increased power of resistance to infection with which they were, presumably, endowed, when the amount of the various substances stood well above the normal, as was the case for the first 4 or 5 months following inoculation. The crucial test of exposure to the actual danger of infection was, however, lacking at the time during which the substances were in evidence in increased quantity on account of the fact that Darjeeling is a healthy hill station, 7,000 feet above the sea level, and no cases of enteric occurred while the regiment was there, with the exception of two men who had not been inoculated, and who contracted the disease when absent at Allahabad.

Further inoculations were carried out on volunteers during the stay of the regiment in the hills, and from among these men fresh groups were constituted upon whose blood

similar tests were done, as opportunity offered, by way of supplement and control of the former results. The number of observations upon these new groups was not sufficient to give more than a general confirmation of the former results, and no new features were brought out. The vaccine employed in these inoculations was the same as had been used at Aldershot, a point of importance, as will be shown later.

After the regiment reached Secunderabad, in the Madras Presidency, cases of enteric fever commenced to appear. The results as regards any evidence of protection conferred by the inoculations were disappointing, but bore out the view that the fall of the protective substances to normal levels coincides with the absence of any degree of increased resistance to infection. The detailed figures in connection with the statistical results of inoculation in this regiment are given in Part II., and it need only be mentioned here that the incidence among the inoculated was practically identical with that among the non-inoculated. The two groups were not, however, homogeneous, as there were, at the end of 1906, only 181 inoculated men in the battalion as contrasted with 832 non-inoculated. As regards the severity and mortality, Captain Smallman reported that the type of the disease in the inoculated was much milder than in the un-inoculated, and that, in his opinion, recent inoculation profoundly modified the severity of a subsequent attack. As regards case-mortality, there was only one death among the inoculated men, and that was in a man who contracted cholera while convalescent from his attack of enteric.

Although the subject is fully dealt with elsewhere, it may further be mentioned, in conclusion, that the comparatively unfavourable results of the inoculation of this unit were the starting point of much of the experimental work described in the following chapters, and that the probable reason for the failure would appear, from the results of these experiments, to have lain in the fact that the vaccine used in this case had been sterilized at a temperature which subsequent experience has shown to damage its immunising properties.

CHAPTER IV.

Experiments on Alternative Methods of Killing the Bacteria.

In this work the investigators kept constantly before them that whatever method was tested should comply with the following requirements: 1st. The method should be simple and certain as regards sterilization and capable of being employed on a large scale. 2nd. The bacteria should show themselves capable of autolysis when kept subsequently at blood heat. 3rd. The vaccine prepared from the bacteria should give rise to a satisfactory development of protective substances on inoculation. 4th. The vaccine should be one which could be standardized with accuracy.

The principle which was adopted in this work was to try a large number of different methods of killing the bacteria and to make a selection of those which gave the most promising results for further tests such as their suitability for accurate standardization and their immunizing properties when used upon experimental animals. There is no need to give details of all the methods investigated and it will suffice to mention those which appeared most promising and the majority of which were submitted to the test of experimental inoculation and the measurement of the protective substances which appeared in the blood of the immunized animals.

Experiment 1.[1]

Object.—To test the value of desiccation as a means of killing the bacteria.

Emulsions of three different strains of typhoid bacilli were made in sterile distilled water and dried in a vacuum over sulphuric acid; sterility was obtained in one experiment in 24 hours, in another not until 72 hours had elapsed. These experiments were, however, performed with but small quantities in watch glasses and when larger amounts were employed, it was found that 6 days might be necessary before complete sterility could be reckoned on. If the emulsions were dried for more than 6 days the dried mass formed by the bacterial bodies was found to be so tough that it could not be emulsified. This change appeared to take place between the 5th and 6th days since, prior to this, the dried mass could be readily emulsified in saline solution. Many attempts were made to overcome this difficulty, for instance, by spreading out the original emulsion in very shallow layers in large dishes,

[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.

but without success. The emulsions made from the bacteria which had been dried for a shorter time than 5 days, although not always sterile, were found to autolyse rapidly when kept for 3 days at a temperature of 37° C., but it would have been necessary to complete the sterilization by filtration at this stage before such a vaccine could be considered safe.

Conclusion.—Desiccation did not appear to be a method suitable for the sterilization of bacteria in the preparation of a vaccine on account of the uncertainty of the destruction of the whole of the germs and of the difficulty of accurate standardization.

Experiment 2.[1]

Object.—To test the value of chloroform as a sterilizing agent.

This reagent was used first in connection with fluid suspensions of typhoid bacteria, either in the form of emulsions of agar cultures or in that of broth cultures of the organism. Such suspensions were placed in flasks and the vapour of chloroform was passed through them by means of a current of air being first drawn through a bottle of the reagent and then through the suspension of typhoid bacteria; air alone was drawn through afterwards in order to free the suspension from the traces of chloroform. The result of a number of experiments of this nature proved that exposure of 1 hour to the action of chloroform vapour could be relied upon to ensure the sterilization of the emulsion or the broth culture. Chloroform was also used to destroy cultures of typhoid bacteria growing upon the surface of agar in large Roux bottles. A few drops of chloroform were poured on the cotton-wool plugs which were then covered with tightly fitting rubber caps, the bottles were then placed in the incubator at blood heat for some hours. This method could usually be relied upon to destroy all the germs on the surface of the agar, but it was noted on one or two occasions that the cultures made to ascertain whether sterilization had been complete, although showing no growth for 2 or possibly 3 days, subsequently gave a growth of pure typhoid. From this it would appear that the latter method was less reliable than the former, and that, at times, some of the germs might be merely inhibited in their multiplication and not actually destroyed, a dangerous possibility in connection with the preparation of a vaccine for use in man.

Bacteria killed in one of these ways by the action of chloroform were next tested as to their potentiality of undergoing autolysis when kept at blood heat for some days. Autolytic action was found to occur readily, but it took longer than in the case of the emulsions prepared from the desiccated bacteria, the same stage not being reached for about 10 days. At the end of this time, however, autolysis appeared to be complete, no recognizable bacteria being encountered in the fluid when this was tested by microscopical examination.

On account of the results of this experiment, and because it was felt that it would not be a matter of great difficulty to overcome the technical troubles of preparing such a vaccine on a large scale, experimental vaccines were made from cultures killed by chloroform and were used to immunise rabbits, whose blood was subsequently examined as to the degree of development of the various protective substances. The results of these experiments are mentioned in Chapter V. (*see* Experiment 8, page 21), and reference to them will show that, while there was a good development of the various substances in the blood of the inoculated animals, they were not superior to those which had been obtained by heated vaccines.

Conclusion.—While it appears that an efficient vaccine may be prepared from cultures which have been killed by chloroform, this method does not present any manifest superiority over other procedures, and the certainty of sterility is not so great.

Experiment 3.[2]

Object.—To ascertain the minimum degree of heat at which typhoid bacteria would be killed, and the comparative value of vaccines prepared from bacteria which had been killed at different temperatures.

Up to the period at which these investigations were commenced anti-typhoid vaccine had always been killed by the action of moist heat, but it was felt that investigations ought to be made to determine the effects of small differences of temperature upon the immunising power of such vaccines. In the past, a temperature of 60° C. was, as a rule, aimed at, and this was controlled by the paraffin thermometer devised by Sir A. E. Wright. This thermometer, however, gave but approximately accurate results and, at times, the temperature of various brews

[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.
[2] *Idem.*

of vaccine exceeded this limit by several degrees. At other times the true temperature was over-estimated with the result that sterility was not obtained, and the vaccine had to be discarded or subjected to a second heating.

The first step was to ascertain with accuracy the minimal thermal death point of typhoid bacteria under the conditions encountered in dealing with a brew of vaccine. A long series of experiments was carried out in this connection, emulsions or cultures being sealed up in glass capsules and completely immersed in a water-bath maintained at the desired temperature. It was found that 53° C. was the minimum temperature which could be relied upon to sterilize a culture of typhoid bacteria in one hour. This observation was repeated many times and with different strains of typhoid bacteria.

As regards autolysis, it was found that cultures which had been killed at this temperature of 53° C. showed a considerable degree, but much less than in the case of cultures killed either by desiccation or by chloroform. On the other hand, cultures which had been exposed to higher temperatures than this showed little or no evidence of autolytic action after incubation for seven days at blood heat.

After determining the lowest temperature which could be relied upon to kill the bacteria in broth cultures or suspensions, a series of experiments was inaugurated to contrast the immunising efficiency of various vaccines which had been exposed to different sterilizing temperatures, but were otherwise identical in their strength and mode of preparation. The results of these experiments are recorded in Chapter V., Experiment 9, and show that, while a vaccine which had been heated at 65° C. is almost inert, one which has been killed at 53° C. is distinctly more efficient in the production of protective substances than a third which has been killed at 60° C.

Conclusion.—A temperature of 53° C. maintained for 1 hour is the lowest that can be relied upon to ensure the sterilization of cultures or emulsions of typhoid bacteria. Vaccines prepared from bacteria which had been killed by exposure to various temperatures, when used to immunise animals, gave the best results in the case of those samples which had been exposed to the lowest temperature.

Experiment 4.[1]

Object.—To ascertain the value of toluol as a sterilizing agent.

The method employed here was to run a layer of toluol, about $\frac{1}{4}$ of an inch deep, on to the surface of a tube containing a culture or emulsion of typhoid bacteria; the tube was then covered with a tightly-fitting rubber cap and placed in the incubator at 37 degs. C., being tested daily for sterility. It was found that all the bacteria died within 3 days. The toluol was then got rid of by simply removing the rubber cap and leaving the tubes in the incubator; it evaporated completely at the end of 2 days. On the fifth day after the addition of the toluol marked autolysis was found to have occurred in the culture, very few unaltered rods being detected on microscopic examination. It was further noted that autolysis was much more rapid and complete with some cultures of typhoid than with others, and that it occurred more rapidly in the case of emulsions made with distilled water than in the case of those made with normal saline solution.

As this method of sterilization would have been simple in application, and appeared to present certain other possible advantages, vaccines were prepared in which toluol was the lethal agent, and were submitted to animal experiment. The results of these have been detailed in Chapter V. (*see* Experiments 10 and 11), where it is shown that the immunizing properties of a toluol-killed vaccine were inferior to those of a heat-killed vaccine, whether a filtrate was employed or the whole vaccine.

Experiment 5.[2]

Object.—To test the value of glycerine as a sterilizing agent.

Emulsions of typhoid bacteria were made in varying strengths of sterile neutral glycerine, and placed in the incubator at 37° C., with the following results:—

100	per cent.	glycerine—sterile	in 24 hours.
40	„	„ „	2 days.
20	„	„ „	4 days.
10	„	„ not sterile	in 8 days.

As the employment of glycerine would also have been a very simple technical method, and as its sterilizing properties appeared to be reliable, this method, too, was submitted to the further test of animal inoculation. The results of the inoculation of

[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.
[2] *Idem.*

rabbits with a vaccine killed by glycerine (Chapter V., Experiment 13) were good, but no better than those obtained with heated vaccines.

A certain amount of autolysis was apparently induced in such glycerine-killed cultures, but it was extremely difficult to estimate on account of the increased transparency of cultures to which large amounts of glycerine have been added.

Glycerine was also employed as the sterilizing agent in the preparation of a vaccine which was used in some of the ingestion experiments recorded in Chapter IX. (*see* Experiment 24).

Conclusion.—Glycerine in strong concentration is a simple and reliable method of killing typhoid bacteria, and a vaccine prepared from bacteria killed in this way was found to be efficient in the production of protective substances in rabbits.

Experiment 6.[1]

Object.—To test the value of alcohol as a sterilizing agent.

Varying proportions of absolute alcohol were added to broth cultures of typhoid bacteria, and, although the results were not constant, it was found that, as a rule, a strength of 10 per cent. of alcohol would sterilize the culture within 24 hours. Examination of cultures or emulsions killed by this amount of alcohol gave no evidence of any trace of autolytic action after incubation of the killed cultures for 7 days at a temperature of 37° C.

It was not thought necessary to inaugurate any immunization experiments with vaccines killed in this way for various reasons, the chief being that the entire absence of any trace of autolysis at blood heat made it probable that bacteria treated in this way would not be readily broken up in the tissues when inoculated, and would thus be inefficient as regards their power of inducing the development of protective substances in inoculated men or animals.

Conclusion.—Alcohol did not appear to offer any advantages as an agent for sterilizing a vaccine.

The general aim of the above series of experiments was to ascertain whether any alternative method of sterilization was preferable to that which had hitherto been employed. Several of the methods described appeared to be effective substitutes for heat, but none showed any decided signs of superiority; while, viewed from the standpoint of the criteria mentioned at the beginning of this Chapter, few could be regarded as satisfactory. With regard to the second of these criteria—that the sterilized bacteria should show some autolysis when kept at blood heat—it appears reasonable to suppose, if the method of sterilization had reacted upon the bacteria in such a way as to render them resistant to autolytic action, as in the case of alcohol, and also when the bacteria were exposed to temperatures ranging above 60° C., that such bacteria would not be as useful for vaccination purposes as those which could be readily broken up and digested by the action of the fluids and cells of the vaccinated man or animal. This view finds some support in connection with the proved efficacy in the case of animals of a vaccine killed at a temperature of 53° C., and the proved inertness of vaccines killed at temperatures ranging from 62° C. to 65° C.

CHAPTER V.

The Relative Efficacy of Vaccines prepared by Different Methods.

It was proposed in this work to prepare various alternative vaccines, and to test the efficiency of these vaccines upon animals. Two means were possible of judging by such animal experiments of the immunizing efficiency of these experimental vaccines: first, the study of the development of protective substances in their blood, and, second, the determination of the degree of protection which such vaccines conferred on animals against multiple lethal doses of virulent typhoid bacteria. A large number of such vaccines were accordingly prepared and tested by the first of these methods, the development of the protective substances in the blood of the inoculated animals. The second method, as is mentioned below (page 32), did not prove satisfactory.

[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.

* In the description of the experimental work which follows it has been thought unnecessary to include the full details, since these have already been published *in extenso* (*loc. cit.*, page 4).

General character of the experiments.

In the great majority of cases rabbits were employed, as they presented many advantages for such a purpose, the chief of these being that they are very responsive to inoculations of typhoid bacteria, whether living or dead, and that they manufacture quantities of the same "protective substances" which are known to appear in the blood of men either suffering from enteric fever or inoculated with a typhoid vaccine. Like men, rabbits were, however, found to show considerable individual differences in respect of the increased quantities of the substances formed in response to inoculation of identical doses of vaccine, and, further, the amounts of these substances were found to vary in different normal animals. This being so, the system of "pooling" the blood of a group of animals, which had been successfully used in the Aldershot experiments, was employed in most of these experiments, and in all instances the tests were carried out not on the blood serum of a single animal but on the pooled serum of a group of animals, to each of which had been given the same dose of vaccine.

Dosage.—Throughout this series of experiments the dosage of the vaccine given to the rabbits has always been calculated in proportion to their weight, relatively to that of a man of average build. Thus, taking the first dose for a man at 500 million bacteria, as fixed from the Aldershot experiments, and taking his weight as being 70 kilos, the dose for a rabbit weighing 2 kgs. would be taken as $\frac{1}{35}$ of this vaccinating dose for a man. Much importance was attached to this proportional reduction in the test doses given to the rabbits, as it secured uniformity of the conditions in this respect, and permitted comparisons being drawn with greater confidence between separate series of experiments. Small as the doses were that were thus employed, it was found that they induced a very definite development of the various substances. The system had the further advantage that, by the use of these small doses, much smaller differences could be detected in the efficiency of different vaccines than was the case if massive doses were employed, as was not infrequently the case in earlier experimental work of this nature.

Except where otherwise stated, the vaccines were administered by hypodermic inoculation, and, as a rule, two or sometimes three doses, increasing in amount, were given at intervals of 10 or more days, unless some reason existed for superimposing them more or less rapidly than this. The blood for examination was drawn under aseptic precautions from the marginal vein of the ear and allowed to clot in blood capsules. The serum was allowed to remain in contact with the clot overnight, and was then pipetted off, and corresponding volumes of the serum of each rabbit in the group were mixed together to form the "pooled serum," with which the various tests, referred to in detail below, were carried out. It was decided, as the result of preliminary experiments, that the most satisfactory method of ascertaining the normal values for the various substances, from which to judge of the degree to which the inoculated animals had responded to the inoculations, was to pool the serum of a "control" group of normal rabbits daily, and, in the case of each analysis, to perform a separate experiment with this pooled normal serum. The result of the various analyses, then, was always read in the light of similar tests applied to the pooled serum of normal individuals, and in this way individual differences were largely eliminated and greater uniformity was obtained than would have been the case if a fixed normal standard had been adopted. Such a fixed standard was further thought inadvisable on the grounds that experiments conducted at considerable intervals of time would be open to fallacies incidental to small variations in technique, in the vitality of the strain employed, and in other points in which it might not always be possible to observe strict uniformity.

Experiment 7.[1]

Object.—To ascertain the influence of the temperature of sterilization upon the immunising properties of a vaccine.

An emulsion of an agar culture of typhoid bacilli, of 24 hours' growth, was made in normal saline solution; this was heated to 65° C., and kept at that temperature for 20 minutes. The emulsion was found to contain 1,283 million germs per ccm., and doses of this vaccine of $\frac{1}{30}$th and $\frac{1}{15}$th of a ccm., respectively, were inoculated into a rabbit at an interval of 11 days.

Tests, similar to those described in connection with the Aldershot experiments, were carried out, either daily or at intervals of two or three days, over a period of one month, to determine the values of agglutinins and bactericidal substances. In this experiment the normal values were not obtained from a control rabbit, as the importance

[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.

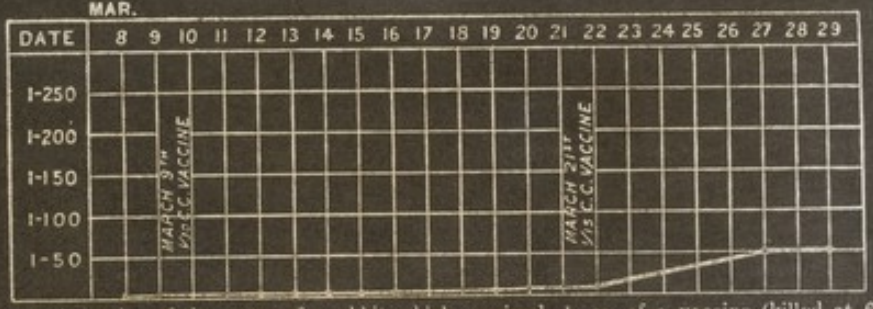
of this was not at the time manifest, and the average of the normal values found by previous experiment was taken as the basis of the various charts.

The result showed that there was no development of bactericidal substances above the normal level, and a very slight and delayed appearance of agglutinins. (*See* Charts VI. and VII.)

CHART VI.

Bactericidal action of the serum of a rabbit which received $\frac{1}{20}$ cc. of a vaccine (killed at 65° C.) on March 9th, and $\frac{1}{15}$ cc. of the same vaccine on March 21st.

CHART VII.



Agglutination value of the serum of a rabbit which received $\frac{1}{20}$ cc. of a vaccine (killed at 65° C.) on March 9th, and $\frac{1}{15}$ cc. of the same vaccine on March 21st.

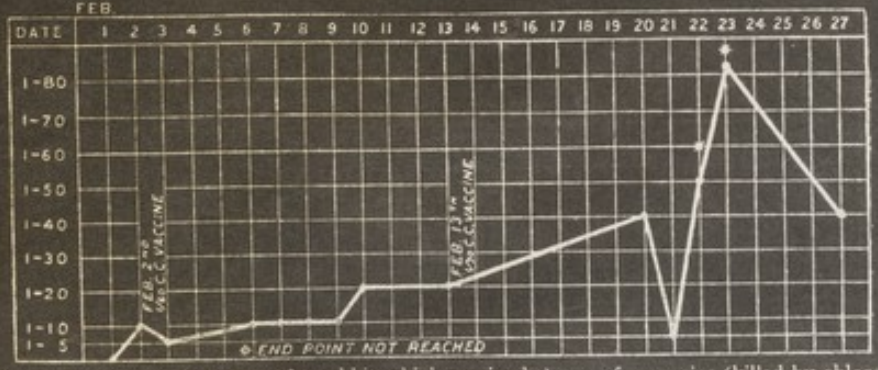
Conclusion.—It would appear from this that heating the bacteria at this temperature of 65° C. gives a vaccine of low efficiency, as judged by the failure of development of the characteristic protective substances.

Experiment 8.[1]

Object.—To test the value of a vaccine which had been killed by chloroform.

An emulsion of a 24 hours' culture of typhoid bacilli was made in normal saline and killed by chloroform (*see* Experiment 2). The vaccine was of the strength of 2,313 million bacteria per ccm. and it was given to a single rabbit, by hypodermic inoculation, in two doses of $\frac{1}{10}$th of a ccm. and $\frac{1}{30}$th of a ccm. at an interval of 11 days. Tests were instituted for agglutinins and bactericidal substances, and the results showed that their development followed the same lines as in the case of men inoculated with cultures killed by heating at 60° C., with the exception that they appeared in the serum somewhat earlier. (*See* Charts VIII. and IX.)

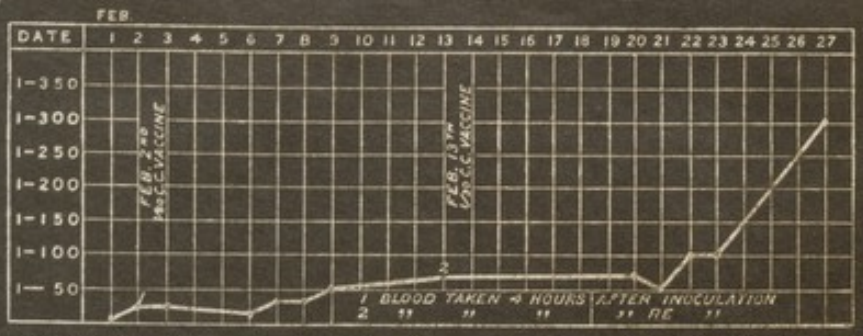
CHART VIII.



Bactericidal action of the serum of a rabbit which received $\frac{1}{10}$ cc. of a vaccine (killed by chloroform) on February 2nd, and $\frac{1}{30}$ cc. of the same vaccine on February 13th.

[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.

CHART IX.



Agglutination value of the serum of a rabbit which received $\frac{1}{10}$ cc. of a vaccine (killed by chloroform), on February 2nd, and $\frac{1}{20}$ cc. of the same vaccine on February 13th.

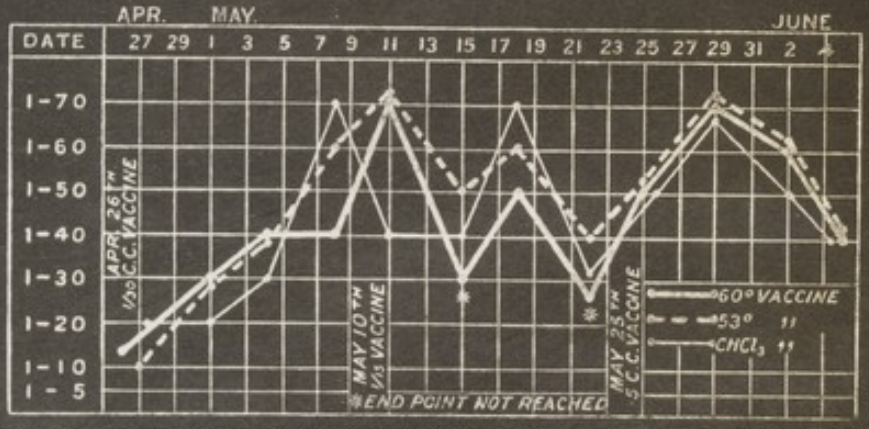
Conclusion.—A vaccine prepared from typhoid bacteria which have been killed by chloroform produces a marked development of protective substances in the blood of an immunised animal.

Experiment 9.[a]

Object.—To compare the efficiency of three vaccines, of the same strength and given in the same doses, which had been killed, respectively, by heating at 53° C., at 60° C. and by the action of chloroform.

An emulsion of typhoid bacteria from a 24 hours' agar culture which contained 1,283 million bacteria per ccm. was divided into three portions; one was killed by chloroform, another by heating at 60° C., and the third by heating at 53° C. An initial dose of $\frac{1}{30}$th of a ccm. was followed, 14 days later, by a dose of $\frac{1}{15}$th of a ccm., and a third dose was given, after a similar interval of 14 days, consisting of $\frac{1}{2}$ ccm. In this experiment groups of rabbits were employed for the first time, and their sera were "pooled" in the manner already described. The system of employing a control group of normal rabbits had not yet been introduced. Tests were carried out as in the case of the two preceding experiments to estimate the fluctuations from day to day of the agglutinins and bactericidal substances. Observations were also made of the phagocytic indices of the pooled sera, which were contrasted with the phagocytic indices of the normal rabbits.

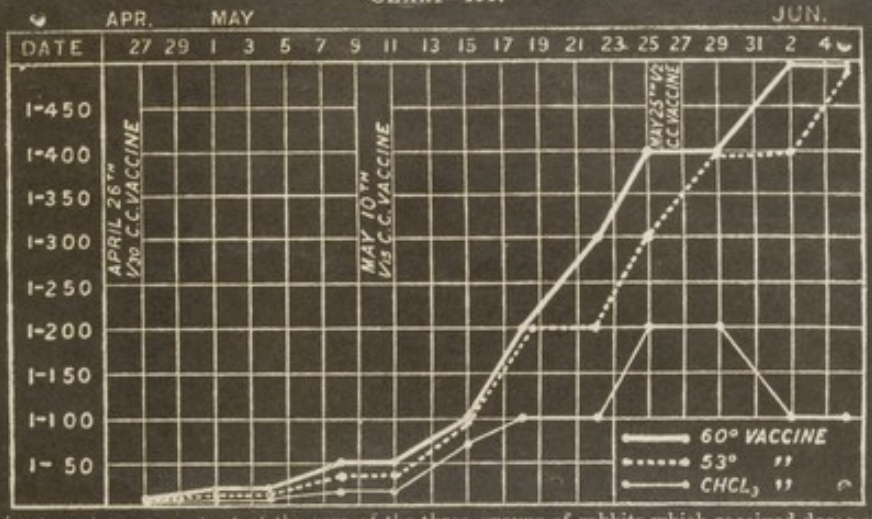
CHART X.



Bactericidal action of sera of three groups of rabbits, which received doses of anti-typhoid vaccine killed (1) at 60° C.; (2) at 53° C.; (3) by chloroform. First dose, $\frac{1}{30}$ cc. on April 26th; 2nd dose, $\frac{1}{15}$ cc. on May 10th; 3rd dose, $\frac{1}{2}$ cc. on May 25th.

[a] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.

CHART XI.



Combined agglutination chart of the sera of the three groups of rabbits which received doses of vaccine, killed by heat at 60° C. and at 53° C., and by chloroform. First dose, $\frac{1}{20}$ cc. on April 26th; 2nd dose, $\frac{1}{15}$ cc. on May 10th; 3rd dose, $\frac{1}{2}$ cc. on May 26th.

The curves of the bactericidal power showed a good development of these substances, no one group appearing to advantage in this respect. As regards the agglutinins, these attained high levels, 1—500 and over, in the case of the rabbits immunised with the vaccines killed at 53° C. and 60° C., but there was a much poorer development in the case of those which received the chloroformed vaccine, 1—200 being the highest point attained. (*See* Charts X. and XI.)

The results of the tests made to contrast the phagocytic index of the pooled serum of each group with that of a group of normal rabbits, the serum employed being heated in each instance, gave the following result. (The experiment was made subsequent to the third inoculation):—

Group.	Average number of bacteria per phagocyte.
Control	2·2
60° C. vaccine	3·1
Chloroformed vaccine	4·4
53° C. vaccine	5·

Conclusion.—While each of the three vaccines in question induced a satisfactory development of protective substances in the blood of the inoculated rabbits, the tests appeared to indicate that the most efficient was that which had been killed at a temperature of 53° C., the chloroformed vaccine coming next. In the case of the vaccine which had been exposed to the temperature of 60° C. the general results were distinctly less favourable.

Experiment 10.[1]

Object.—To ascertain whether the filtrate from a culture of typhoid bacilli which had been killed by toluol and had undergone considerable autolysis possessed any immunizing properties for rabbits.

An emulsion was made by adding 1 ccm. of saline solution to each of a series of agar tubes of typhoid; the culture employed in this instance was a virulent one. Toluol was added to the emulsion thus obtained and it was then allowed to autolyse and finally filtered through a Kitasato candle. The filtrate, after having been proved to be sterile, was inoculated into two rabbits, 1 ccm. being given hypodermically as a first dose, followed, 24 days later, by a second dose of 2 ccm.

In this experiment, and in all subsequent observations of similar nature, a control group of normal rabbits was formed and analyses were performed upon the pooled blood of this control group on each occasion that the individual tests were carried out upon the pooled blood of the inoculated group. The readings obtained daily from the normal group furnished the normal standard from which to gauge the results obtained in the case of the immunized animals, the curves recorded in the charts being framed on new lines in accordance with this system.

[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472 1907

Tests were made over a period of one month as to the development of agglutinins and bactericidal substances.

The first named were late in appearing and never reached a higher level than 1—60, but, at the conclusion of the experiment, they appeared still to be rising as the result of the second dose. Little effect was produced upon the bactericidal power of the serum, indeed, it showed a tendency to decline to and below the level of that of the control group during the course of the experiment, but at its termination it was slowly recovering.

Conclusion.—The filtrate from the autolysed culture of typhoid bacteria which had been killed by toluol, although not inert, produced but a feeble development of protective substances when employed to immunise rabbits.

Experiment 11.[1]

Object.—To contrast the immunising efficiency of two vaccines, one of which had been killed by chloroform and the other by toluol; neither was filtered before use.

Rabbits were immunised in the same way and with the same doses as were given in the case of Experiment 10, above. Bactericidal substances only were tested for on this occasion and the daily estimations were controlled by a similar series of estimations upon the blood of a normal group of animals.

The result showed that the development of the bactericidal substances, in the case of the group inoculated with the chloroformed culture, followed the same curve as in the case of the previous experiments done with this vaccine (*see* Experiments 8 and 9); on the other hand, the inoculations of the unfiltered toluol vaccine were followed by a gradual and progressive decline of the bactericidal power, until, finally, the serum of the group showed no bactericidal power at all. It was thought possible that the toluol vaccine might have produced a prolonged negative phase by reason of the extensive autolysis of the bacteria, but this explanation is hardly probable, since the inoculations of other vaccines, in which the method of preparation had induced a degree of autolysis in no degree inferior to that effected by the toluol, were not followed by a prolonged negative phase of bactericidal power.

Conclusion.—The toluol-killed vaccine was of no immunising value, as judged by the negative phase of bactericidal power which it induced; that which was killed by chloroform, on the other hand, produced a satisfactory development of this power, and confirmed the results of the preceding experiments.

The 11 experiments briefly described above were, approximately, of about the same period and, taken in conjunction with much incidental work in other directions, some of which is detailed below, they led Sir William Leishman and his colleagues to the conclusion that the temperatures formerly employed for the sterilization of the vaccine had occasionally been too high. It was evident, for instance, that a vaccine heated to 65° C. had lost a great part of its power to induce the formation of protective substances in inoculated animals. The experiment (No. 9) in which the effects of vaccines heated to 60° C. and 53° C., respectively, were contrasted, brought out the same point, namely, that the advantage lay with that which had been heated at the lower temperature. The experiments with the other vaccines killed by alternative methods, although satisfactory in some instances, such as in the case of that killed by chloroform, had not shown to their conviction that they were superior to a vaccine killed by heat.

They decided, therefore, that the vaccine should thenceforth be killed by heating for one hour at a temperature which was not allowed to exceed 53° C., this being the point which they had experimentally fixed as the minimum thermal death-point of the typhoid bacillus within this period of one hour. The Committee fully approved of this action, as recorded in the minutes of the 12th meeting held on 13th October, 1905, and since then no vaccine has been issued for use by the troops which has been subjected to a temperature higher than 53° C.

It appears possible that some of the irregular results which have been recorded in the past, in the case of the vaccines formerly employed, may have been due to the fact that some batches of vaccine had been superheated in manufacture, and had thus lost some of their efficacy, either as regards the degree or the duration of the protection which resulted from their use.

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[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.

Experiment 12.[1]

Object.—To contrast the efficiency of a vaccine which had been killed by heating at 53° C. with that of one which had been killed by the action of lysol.

This experiment was instituted in order to ascertain whether the customary addition of a small amount of antiseptic to the vaccine after heating had any deleterious effect upon its immunising properties. Besides this, it was thought possible that killing the germs by the action of lysol alone, without any previous heating of the vaccine, might result in the production of a more potent vaccine. The amount of lysol added to the vaccines was 0·25 per cent., and experiment had shown the reliability of this small amount to ensure the destruction of any germs which might subsequently gain entrance.

A 48 hours' broth culture of typhoid was divided into two parts; one was killed in the customary manner by being heated at 53° C. for one hour, 0·25 per cent. of lysol being added after this had been done. The other half received the same addition of lysol but without the culture having been heated beforehand. Both vaccines were found to be sterile at the end of 24 hours.

Groups of rabbits were then inoculated with each vaccine, identical doses being given in each instance, and quantitative tests were commenced on the pooled serum of each group, control experiments being carried out simultaneously with the pooled serum of a group of normal rabbits.

It was found, in the case of the group which had been inoculated with the vaccine killed by the action of lysol only, that there was a definite rise of agglutinins and bactericidal substances, while the phagocytic index was also raised in response to the inoculations. On the other hand, no trace of any rise in these substances occurred in the group inoculated with the heated vaccine, to which the same amount of lysol had subsequently been added.

This result was unexpected, as vaccines, apparently identical, had been frequently tested on animals with excellent results as regards the development of protective substances. It was conjectured that the inactivation of this vaccine might possibly have been caused by the fact that, on this occasion, the lysol had been added before the vaccine had been allowed to cool down after removal from the water-bath in which it had been sterilized. To test this a second series of observations was carried out, the heated vaccine this time being cooled down to room temperature before the lysol was added. The results showed that the above conjecture had been correct since, in this instance, there was the customary good development of all the protective substances for which tests were made, not only in the case of the lysol-killed portion but also in that of the heated portion. As regards the relative value of the two no essential distinction could be made between them, the values recorded in the case of the various substances being approximately the same and the curves more or less alike; both showed an effective immunising power.

Conclusions.—First, it appears unnecessary to heat the vaccine beforehand, since the subsequent addition of the lysol may be relied on to sterilize it efficiently without apparently diminishing its value as a vaccine; second, previous heating of the vaccine to this temperature of 53° C. does not lessen its efficacy as a vaccine, when contrasted with one which had been killed by lysol alone.

As no apparent advantage was found to lie on either side, it was considered unnecessary to make any alteration in the system of heating the vaccine and of subsequently adding the necessary amount of antiseptic. It was, however, recognised that this experiment had also served to bring to light a possible explanation of the failure of certain batches of vaccine used in the old days. It was not at that time suspected that the addition of the antiseptic to the vaccine before it had cooled down could have any deleterious effect upon its properties, and at times of pressure this was occasionally done, although in most cases, as they were informed by Sir William Leishman who made most of the vaccine used in the South African war, under the directions of his then chief, Sir A. E. Wright, it had been allowed to cool down before this addition. Needless to say, this precaution has always been observed since this experimental proof of its necessity was realised.

Experiment 13.[1]

Object.—To determine the efficacy of vaccines which had been killed by glycerine.

The experiments made in connection with various alternative methods of killing the bacteria (*see* Experiment 5) having shown that certain advantages attached to the use of glycerine for this purpose, it was decided to test such a vaccine by inoculating groups of

[1] Journal of the Royal Army Medical Corps, Vol. X., page 583, 1908.

rabbits in the manner described above. Two such vaccines were prepared from strains of different virulence.

Broth cultures of each strain were grown for 24 hours, and to each was then added 20 per cent. of sterile pure glycerine. The cultures were kept at blood heat for a week, and at the end of this time they proved to be sterile. The bacterial content of each culture was approximately the same, 503 million in the case of the non-virulent culture, and 475 million in that of the virulent strain. Each group of rabbits received $\frac{1}{25}$ of a ccm. for the first dose, $\frac{1}{1\frac{1}{2}}$ ccm. for the second, and $\frac{1}{6}$ ccm. for the third; 16 days separating the doses. Tests were carried out for agglutinins and bactericidal substances, while the phagocytic indices of the heated serum were also ascertained. In all cases the results of these tests were read in the light of control observations upon the pooled serum of a third group of normal rabbits. The test organism employed in the various experiments was the non-virulent strain, from which the non-virulent glycerine vaccine had been prepared.

The experiment was continued throughout 2 months. During this time there was a very poor development of agglutinins, the highest point reached—and this on one occasion only, and in the case of the virulent vaccine—was 1-60. As regards the development of bactericidal substances, little effect was noted in the case of the two first inoculations, the values being very little higher than those recorded in the normal animals, but after the third dose there was a distinct increase in the case of the virulent vaccine, while the non-virulent was not much higher than the controls. The tests made for the substances concerned in phagocytosis did not show a very marked elevation in the amount of these substances; such as it was, however, it was in favour of the vaccine which had been prepared from the virulent strain. It will be recalled that these tests were carried out with the non-virulent bacilli which had been used in the preparation of one of the vaccines, so the results in favour of the virulent one have more significance than would otherwise have been the case.

Conclusion.—Vaccines prepared by killing the typhoid bacilli with glycerine, although active, do not appear to have a high immunising value. That which had been made from a virulent strain of bacilli appeared somewhat more active in the production of immunising substances than that made from a non-virulent strain. (For other experiments upon the relative value of virulent and non-virulent cultures, *see* Experiment 17, page 31.)

Experiment 14.[2]

Object.—To ascertain the influence of the age of a vaccine upon its immunising properties.

For the purpose of investigating this important point two vaccines were made use of. Both had been prepared from the same strain of typhoid bacilli; the method of preparation and sterilization had been identical, and the same doses were given, as described below, to groups of rabbits. The two vaccines differed only in respect of their age and of the alterations of temperature to which the older of the two had been exposed. (The influence *per se* of such alterations of temperature formed the subject of another series of experiments. *See* Experiment 16, page 30.) One of the vaccines had been freshly prepared, but the other was a year old and had been returned to the laboratory after having been out to New Zealand and back. There was no apparent alteration in the older vaccine, and it was sterile when tested.

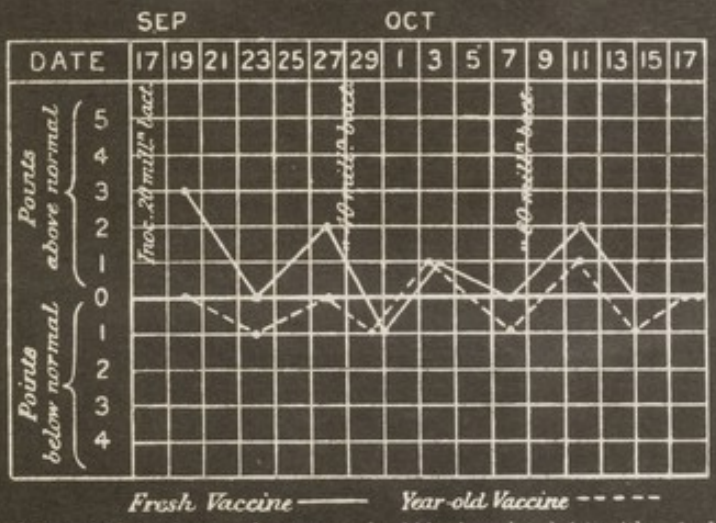
Two groups of rabbits were inoculated with identical doses of each vaccine, containing, respectively, 20, 40, and 80 million bacteria, at intervals of 11 days between first and second doses, and 9 days between second and third. A third group of rabbits was not inoculated, but furnished the pooled normal blood upon which similar tests were carried out daily.

In this experiment, which took place at a date considerably later than those detailed above, the tests were four in number: agglutinins, bactericidal substances, the estimation of the phagocytic indices, by Wright's method and by Klien's dilution method.[3] Tests which had been made in connection with the latter method in the laboratory had shown that it possessed certain advantages over the older methods of Leishman and of Wright. The two methods were, therefore, employed in this and in some of the later experiments, as the general trend of the experimental work had been to emphasize the importance of the changes in the blood in respect to phagocytosis in the course of immunisation against typhoid bacteria.

[1] Journal of the Royal Army Medical Corps, Vol. X., page 583, 1908.
[2] *Idem*, Vol. XI., page 327, 1908.
[3] Johns Hopkins Bulletin, Vol. XVIII., page 245, 1907.

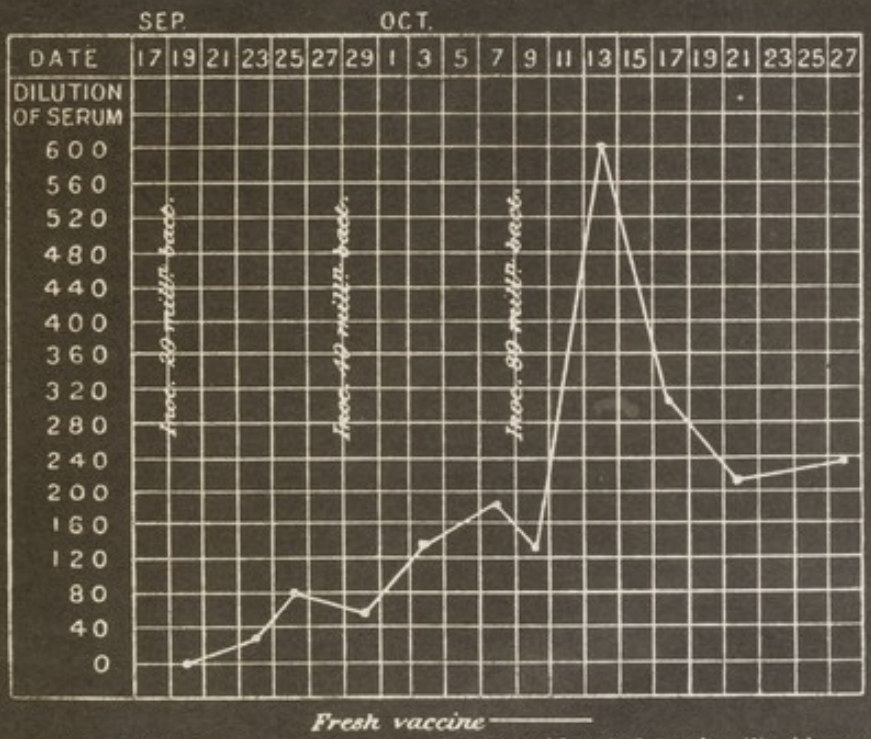
The bactericidal substances formed in the blood of the inoculated rabbits were measured quantitatively on each occasion, and the result contrasted with a similar observation made upon the pooled serum of the normal group. The result showed that there was little to choose between the fresh and the old vaccine in this respect, the curves of these substances following rather an irregular line; such as it was, the advantage lay with the vaccine which had been freshly prepared. (*See* Chart XII.)

CHART XII.



Development of *bactericidal substances* in groups of rabbits inoculated (*a*) with a *fresh* vaccine, (*b*) with one *a year old*.

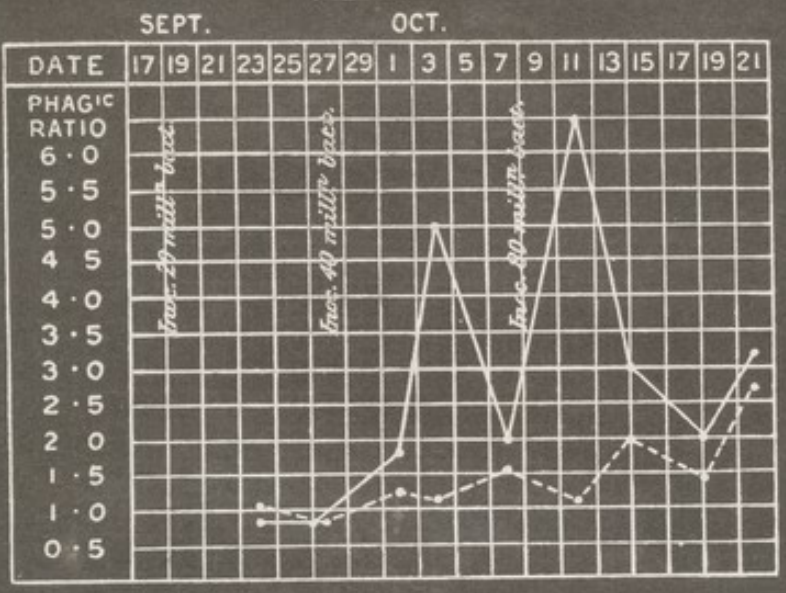
CHART XIII.



Development of *agglutinins* in groups of rabbits inoculated (*a*) with a *fresh* vaccine, (*b*) with one *a year old*.
NOTE.—*No agglutination* over 1-10 was noted in the rabbits inoculated with the old vaccine.

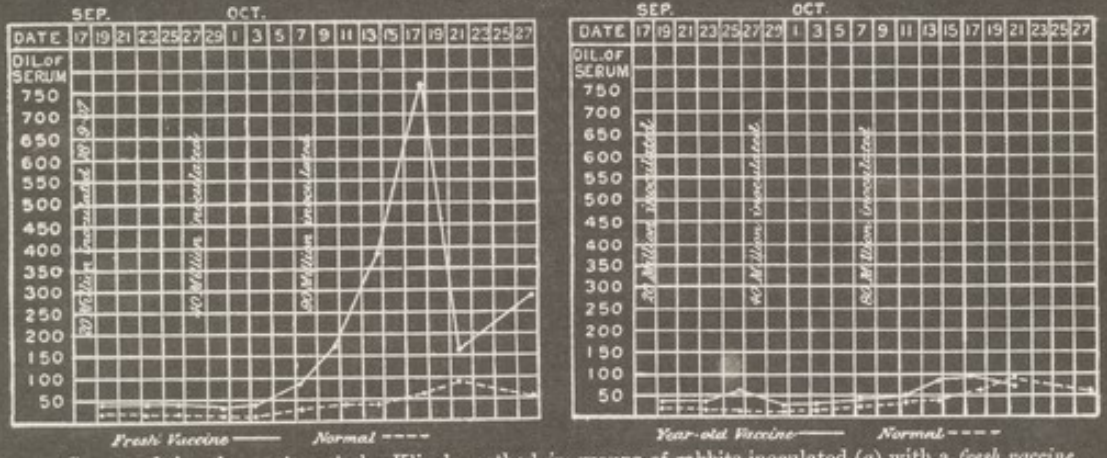
Tests for the agglutinins, on the other hand, brought out a very striking result; while the curve for the rabbits inoculated with the fresh vaccine followed the customary lines and, subsequent to the third dose, the value in this group rose to 1—600, the rabbits who had been inoculated with the year-old vaccine failed altogether to show any response to the inoculations. The highest value recorded was a trace of agglutination in a dilution of 1—10. The normal rabbits gave negative results throughout. (*See* Chart XIII.)

CHART XIV.



Curves of the *phagocytic ratios*, by Wright's method, in groups of rabbits inoculated (*a*) with a *fresh vaccine*, (*b*) with one *a year old*.

CHART XV.



Curves of the *phagocytic ratio*, by Klien's method, in groups of rabbits inoculated (*a*) with a *fresh vaccine*, (*b*) with one *a year old*.

The experiments carried out to test the development of the phagocytic substances gave a similar result; both methods showed that that the old vaccine had but a slight influence in increasing these substances, while the fresh produced them in large amount. (*See* Charts XIV. and XV.)

Conclusion.—The vaccine, which was a year old and had voyaged round the world, appeared, from the above tests, to have lost a considerable amount of its immunising properties when contrasted with a fresh vaccine.

As the above conclusion had an obvious importance in connection with the practical application of the method of inoculation the experiment was repeated.

Experiment 15.[1]

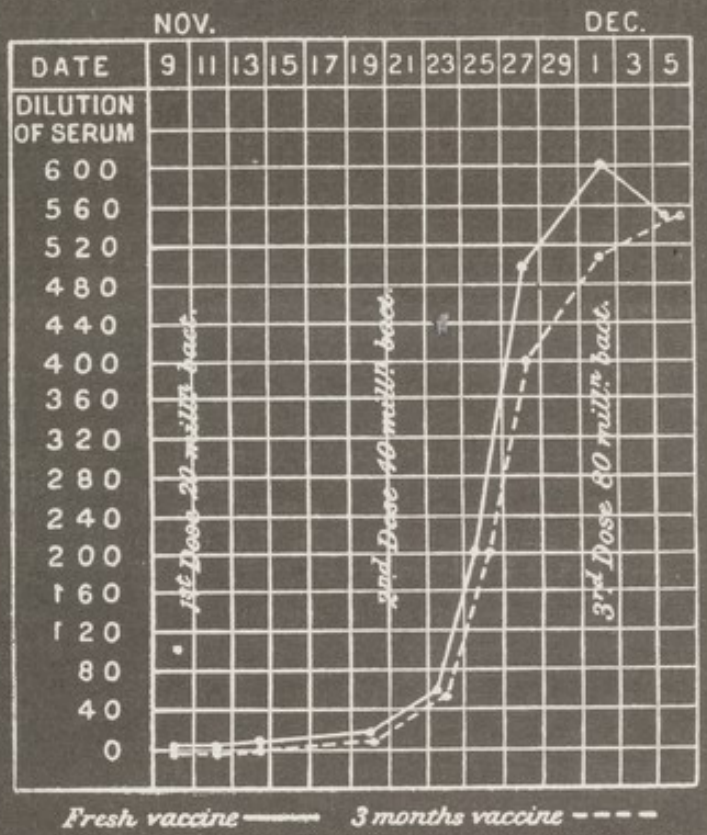
Object.—To ascertain the influence of age upon vaccines which had been kept under identical conditions.

On this occasion three vaccines were made the subject of experiment. All had been prepared from the same strain, and the details of their preparation had been as closely identical as possible. All of the vaccines had been kept in the same cupboard at room temperature from the date of their preparation. Their ages, on the day of the commencement of the experiment, were, respectively, 6 days, 11 weeks and 5 days, and 27 weeks. (They will be alluded to below as the "fresh," the "3-months'," and the "6-months'" vaccines.)

[1] Journal of the Royal Army Medical Corps, Vol. XI., page 327, 1908.

In this case, therefore, four groups of rabbits were employed, three being inoculated with the respective vaccines, the fourth serving as the control. The inoculated groups received three doses, 20, 40 and 80 million bacteria, respectively, at intervals of 10 days in each case. Tests were made for agglutinins and for the phagocytic substances, the latter by both Wright's and Klien's methods. Bactericidal substances were not included in this investigation, chiefly because of the great labour involved in making so many quantitative estimations daily.

CHART XVI.

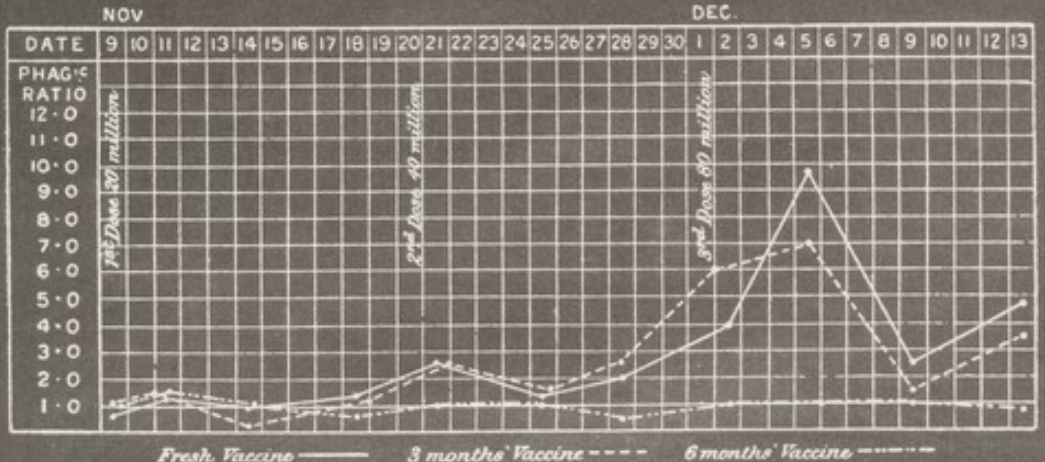


Development of *agglutinins* in three groups of rabbits, inoculated, respectively, with *fresh vaccine, three months' vaccine*, and *six months' vaccine.*

NOTE.—*No agglutinins* noted in the rabbits inoculated with the vaccine *six months old.*

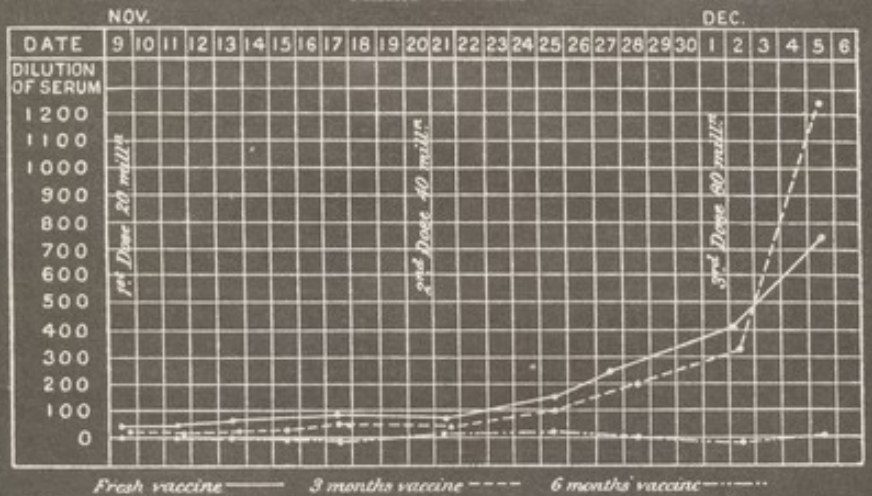
The agglutinin curves once more brought out in emphatic fashion the contrast between the vaccines of different ages. While there was practically no difference in the values of the fresh vaccine and the 3-months' vaccine, the development of agglutinins in both being normal and abundant, on the other hand, no trace of these substances could be detected throughout the whole period of the experiment in the case of the pooled serum of the group inoculated with the 6-months' vaccine. (*See* Chart XVI.)

CHART XVII.



Curves of the *phagocytic ratios*, by Wright's method, in three groups of rabbits, inoculated, respectively, with *fresh vaccine, three month's vaccine*, and *six months' vaccine.*

CHART XVIII.



Curves of the *phagocytic ratios*, by Klien's method, in three groups of rabbits, inoculated, respectively, with *fresh vaccine*, *three months' vaccine*, and *six months' vaccine*.

The above result was borne out in a striking manner by the tests made in connection with the phagocytic activity of the blood of the immunised rabbits. Both testing methods yielded similar results, namely, a good development of these substances in the case of the fresh and the 3-months' vaccines, little difference existing between the two in this respect; but there was no evidence of increased phagocytic activity in the blood of the rabbits inoculated with the 6-months' vaccine, indeed, their blood, in this respect, could not have been distinguished from that of the non-inoculated group of control animals. (*See* Charts XVII. and XVIII.)

Conclusion.—The 3 months' old vaccine appeared to be as effective as that which had been freshly prepared, but the vaccine which was 6 months old gave rise neither to agglutinins nor to the substances which influence phagocytosis, and, on this account must be judged to have lost at least a part of its immunising efficiency.

It will be seen that the results of Experiment 15 confirmed those of Experiment 14, and together they proved, as far as this could be done by laboratory experiment, that a vaccine which is 6 months old may have lost by that time at least a part of its immunising efficiency. While this deterioration must, therefore, be looked upon as a possibility, in the case of vaccines of this age or older at the time of their use, it is quite possible that it is not the case in all instances and, indeed, there is some evidence from the statistical point to show that vaccines older than this may afford a certain degree of protection against actual infection. However this may be, on the conclusion of these experiments Sir William Leishman and his colleagues at once took steps to ensure that no vaccine of greater age than 3 months should be employed for inoculation, and instructions to this effect were printed upon the labels of each phial of vaccine and circulated to all concerned in its distribution. On reporting his action to the Committee at their next meeting, this step was fully approved.

This question of the age of the vaccine may present a further explanation of the comparative failure of the system in certain instances. Before the results of the experiments just quoted were arrived at, it was not realised how rapid and extensive might be the deterioration of the vaccine through age. Although that issued in the past 3 or 4 years for use on troops at home has, in the great majority of instances, been no older than would appear from the above to be the safe limit, it is certain that, at times, and especially in India, vaccines have been used older, sometimes very much older, than 3 months. In the light of the above experiments, it may be assumed that the degree of protection given by such old vaccines was very slight. This assumption finds support in some of the recent statistical returns, and will be referred to again in Part II., which deals with this branch of the subject.

Experiment 16.[1]

Object.—To ascertain the influence upon the immunising qualities of a vaccine of the temperature at which it has been stored.

This enquiry was suggested by the results of Experiment 14, in which it was found that a vaccine which was a year old, and had been out to New Zealand and

[1] Journal of the Royal Army Medical Corps, Vol. XI., page 327, 1908.

back, had lost a large part of its immunising properties. During its voyage, this vaccine had, of course, been exposed for a considerable period to tropical heat, and it was not possible to determine whether the heat or the age-factor had played the predominating part in this deterioration. It is true that in Experiment 15 the different vaccines had been kept under identical conditions as regards temperature, but in this case they had naturally been subjected to this temperature for very different periods, so it was still doubtful whether such a temperature as vaccine may be exposed to in the tropics has a deteriorating influence upon it. The following tests were, therefore, made to attempt the experimental elucidation of this important point.

After a vaccine had been prepared in the customary manner by sterilization at 53° C., three samples of it were set aside, hermetically sealed in glass phials, for a period of 3 months. All were kept in the dark and not disturbed in any way until the commencement of the experiment. One was kept in a cupboard at room temperature, which averaged 16° C., the second was kept at blood heat in the 37° C. incubator, and the third in the ice chest at a temperature a few degrees above the freezing point.

At the commencement of the 4th month from the date of preparation of the vaccine a series of inoculations was carried out, on the lines of the preceding experiments, upon groups of rabbits whose blood was pooled and tested from day to day. In this case, however, the animals, with the exception of the usual control group, received in all 4 doses, of 20, 40, 80 and 160 million bacteria respectively, at intervals of from 11 to 14 days. Their phagocytic power was tested by both Wright's and Klien's methods and the agglutinins were estimated; no tests were carried out for the bactericidal substances, for the reasons mentioned in the foregoing experiment.

The results of this experiment, which extended over 7 weeks, may be briefly summarized. The vaccine which had been kept at room temperature yielded the best results, but neither of the others showed a very marked inferiority. Neither of those kept at the extremes of temperature was by any means inactive, but both appeared to have lost some portion of their immunising properties, as contrasted with that which had been kept at room temperature and also as contrasted with the results of former experiments on rabbits with vaccines of about the same age which had also been kept at room temperature.

Conclusion.—It would appear from the above that it is desirable to keep vaccine at a moderate temperature and that extremes, either of heat or cold, are to a certain extent deleterious and to be avoided. (The effect of keeping vaccine in a refrigerating room, a suggestion which has sometimes been made with a view to the preservation of its immunising properties, is however being further tested.)

Experiment 17.[1]

Object.—To ascertain the influence of the virulence of the strain of typhoid bacteria upon the immunising properties of a vaccine.

The results recorded in Experiment 13, in which two vaccines made from cultures killed by the action of glycerine, the one a virulent, the other a non-virulent strain, showed that there was an apparent advantage in that instance in the use of the virulent vaccine, as the protective substances on the whole appeared to be developed in greater amount than in the case of the animals inoculated with the non-virulent vaccine. This point was, therefore, once more put to the test by preparing two vaccines with strains of different virulence and inoculating with these vaccines two groups of rabbits. On this occasion the vaccines were prepared in the usual manner by sterilization at 53° C., since the fact of the former vaccines having been killed by glycerine might have introduced new factors complicating the conclusions drawn from Experiment 13.

The non-virulent strain used in this experiment was that in general use for the preparation of the vaccine issued to the Army and had been sub-cultured through innumerable generations during the 4 years which had elapsed since it was isolated from a fatal case of typhoid fever. The virulent strain, on the other hand, had been recently isolated from the blood of a case of typhoid fever and, at the time of the experiment, was found to be lethal to a guinea-pig of 250 grammes weight on the intraperitoneal inoculation of 0·5 ccm. of a 24 hours' broth culture.

Each group of rabbits received in all three doses of these respective vaccines, containing respectively 40, 80 and 160 million bacteria, the interval between the first and second doses being 14 days, and between the second and third, 25 days. A third group

[1] Journal of the Royal Army Medical Corps, Vol. XI., page 327, 1908.

of normal rabbits served, as usual, to furnish the pooled serum to control the estimations made upon those inoculated. Before beginning the experiment several observations were made upon the blood of the various groups, the result showing that there was no essential difference between them, thus furnishing additional evidence of the reliability of the quantitative methods employed.

The agglutinins and the phagocytic ratios, the latter by both Wright's and Klien's methods, were determined. The agglutinins showed the usual course of development in both of the inoculated groups and the levels attained were high in each instance, the curves showed almost identical fluctuations, rising and falling at the same time, and, at the conclusion of the experiment, 52 days after its commencement, they stood at practically the same heights. The strain of typhoid bacteria employed to test the agglutinating power of the pooled sera was non-virulent, but not that from which the non-virulent vaccine had been prepared.

As regards the phagocytic ratios these responded readily in each instance to the respective doses of the vaccines, and, in this instance also, it was not possible to assign a greater value to one vaccine than to the other; if one appeared to show to advantage at one time the balance inclined to the other side at a later stage. The test organism in this case was a virulent one which had been recently isolated from a fatal case at Bermuda, but was not that employed in the preparation of the virulent vaccine.

Conclusion.—It was gathered from the results of this experiment that the inoculation of a vaccine prepared from a non-virulent strain of typhoid bacteria was capable of inducing as satisfactory a development of "protective substances" in the blood of inoculated animals as one prepared from a strain of high virulence.

From this experiment it will be seen that the impression left by the results of Experiment 13 had not been confirmed, and for this and other reasons it was decided not to make any alteration in the system then in force of making the vaccine for the troops with a strain of little or no virulence.

As was stated at the commencement of this chapter it was intended to test the efficiency of these experimental vaccines, not only by the estimation of the protective substances which appear in the blood after inoculation but also by contrasting their immunising value for animals by determining the degree of protection which was afforded by such vaccines against multiple lethal doses of virulent typhoid bacteria.

Experimental inoculations in connection with this second method were therefore undertaken in the case of some of the vaccines first experimented with, but they were eventually abandoned on account of the fact that they proved incapable of detecting such small differences in the immunising properties of different vaccines as the quantitative estimation of the protective substances. The principal reason for this lay in the fact that the animals experimented with displayed such variations in their respective powers of resistance to infection, even when of the same age and inoculated with identical doses, that the only method by which certainty could be secured would have been the inoculation of a very large number of animals in each experiment and, unless the results were likely to be of great importance, this hardly appeared justifiable. Besides this there was a great difficulty in maintaining a particular strain of typhoid bacilli at a constant degree of virulence and here again the individual resistance of different animals rendered the task of estimating the exact lethal dose of the strain a matter of uncertainty. Further, although it might have been possible, by the use of a large number of animals, to get fairly good comparative results during one experiment, there would have been great difficulty in adjudicating the results obtained in another experiment, conducted with the same strain some months later. The employment of a fresh strain would have been almost a necessity and, once the strain had been changed, a number of factors would have been introduced into the problem which would have made it almost impossible to draw definite conclusions as to the superiority or inferiority of the vaccines being tested.

Some of the experiments done in this connection are briefly described below.

Experiment 18.[1]

Object.—To ascertain the protective action of three different vaccines, killed, respectively, by heating at 53° C., by heating at 60° C., and by the action of chloroform, against multiple lethal doses of a virulent typhoid culture.

The culture employed in this experiment was first tested as to its virulence and it was determined that a dose of 0·5 ccm. of a 24 hours' broth culture, per 250 grammes of guinea pig, when given intra-peritoneally, could be relied upon to kill a guinea pig within 24 hours.

[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.

A group of three guinea pigs was vaccinated with the three different experimental vaccines, which were identical in strength, and given in doses of 0·1 ccm. per 100 grammes of guinea pig. A test dose of the virulent culture, containing twice the quantity which had been proved to be lethal within 24 hours, was given to each guinea pig of the particular group, by intra-peritoneal inoculation, 27 days after they had received their immunising dose of vaccine. A control animal which had not been inoculated received a similar dose of the virulent bacteria.

In this instance the control animal died in 21 hours, while the whole of the inoculated animals survived, so no conclusion could be drawn as to the relative value of the different vaccines.

The next series of experiments, a lengthy and laborious test, was completely invalidated by the failure of the test dose of living culture to kill the control animals, the virulence having in the mean time fallen off.

In the following series the test dose, as the result of incidental experiment, was derived from broth inoculated direct from an agar stab, which had been kept hermetically sealed. Three lethal doses of the culture were given 11 days after the inoculation of the various groups. This time the whole of the animals died, with the exception of one of those which had been inoculated with the vaccine killed by chloroform. Typhoid bacteria were recovered in pure culture from the heart blood of the animals which succumbed.

In the next series the test dose of culture was reduced to two lethal doses, in the hopes of eliciting more definite information as to the respective immunising properties of the different vaccines, and it was administered 12 days after vaccination. The whole of the vaccinated animals survived, the control dying in 20 hours with typhoid bacteria in its blood. Once more no evidence other than that of the common protective value of the different vaccines was obtained.

In another series the animals were given two doses of 0·2 ccm. of vaccine per 250 grammes weight, at an interval of 11 days, and this was followed, after a similar interval, by four times this amount of vaccine. Two lethal doses of culture were given to the animals of each series 13 days after the last inoculation. The control animal died in less than 20 hours, while the whole of the inoculated animals survived.

In the last experiment which need be quoted the animals were vaccinated in the same manner as in the last series, but received 10 lethal doses 14 days after their third dose of vaccine, the control animal receiving, as usual, a single lethal dose. Here, again, the whole of the inoculated animals survived, while the control died within 24 hours, typhoid bacilli being isolated from its heart blood in pure culture.

Conclusion.—Each of the three vaccines proved capable of protecting a guinea pig against two minimal lethal doses of a virulent culture of typhoid bacteria after the subcutaneous inoculation of 0·1 ccm. per 100 grammes body weight of the particular vaccine, while, if the animals were reinoculated, they were able to resist 10 lethal doses. No conclusive evidence, however, was obtained as to the relative efficiency of the vaccines killed respectively at 53° C., at 60° C., and by the action of chloroform.

Doubtless, as has been said, by multiplying the number of animals and by conducting simultaneous experiments with the test-strain of virulent bacteria, one might be able to elicit differences in respect of the protective value of a series of vaccines; but, for many reasons, this method was abandoned as being too cumbrous and uncertain for the purpose which was in the view of the investigators. Evidence of the protective value of the inoculation of dead cultures of typhoid bacteria is clear from the above-quoted experiments, but this has already been abundantly proved by the numerous experiments of similar nature which have been conducted in other laboratories for years past.

CHAPTER VI.

Experiments bearing on the Technique of Preparation of the Vaccine.

The experimental work which has been carried out in this connection has, naturally, been considerable and continuous. Much of it, however, although of great importance from the point of view of facilitating the preparation of the vaccine upon a large scale and of safeguarding it from contamination during the process of manufacture, is concerned with details of procedure and technique which it appears unnecessary to include in the Report. A few of the experiments are, however, given in illustration of

some of the more important of the investigations. The points selected are, respectively, the conditions which were found to favour the growth of the typhoid bacilli to the greatest extent, the mode of applying the temperature necessary to kill the bacilli and the nature and strength of the antiseptic to be added to the vaccine after sterilization. It may be added that these investigations are only such as were directly connected with the vaccine which has been adopted and which is now in use; details of work on similar lines, in connection with vaccines prepared in other ways, is not included.

Experiment 19.

Object.—To study the conditions under which optimum growth of typhoid bacilli is obtained.

Having previously determined to employ none but young cultures of typhoid bacteria in the preparation of a vaccine it was of obvious importance to secure maximum growth of the bacteria within the time laid down, namely, 48 hours. This was demanded on account of the importance of economising time and material in the preparation of vaccine on a large scale and because a small dose, as regards total volume of fluid injected, is preferable to a large one. Poor growths of typhoid in the various culture media yield but a small amount of vaccine, as measured in terms of the number of bacteria, and, if the medium selected be nutrient broth, a quantity corresponding to 2 to 5 ccm. might be required for each inoculation.

Without going into needless details as to the many experiments carried out in this connection, it may be shortly stated that the more important of the factors which were found to influence the rapid and vigorous growth of typhoid bacteria in broth appeared to be the following:—

1. The nature of the strain employed.—It was soon found that all strains did not behave alike when grown under identical conditions. It was not clear upon what this variability depended as no constant factor such as age, virulence, length of time since solation, &c., was found. The fact, however, being recognized, selection has been made of a strain which was found to give good results in this respect from among those which answered the other requirements for the preparation of a vaccine. Certain strains, too, besides being possibly inferior in their powers of rapid multiplication, were also found to be unsuitable for use in a vaccine on account of their tendency to auto-agglutinability, such a tendency would of course greatly increase the difficulties of any method of standardization which depended upon the enumeration of the actual number of germs present in a given volume, if it did not necessitate the employment of an altogether different method.

2. The reaction of the culture medium.—It was found, as has often been noted in the case of other bacteria, that typhoid bacilli were extremely sensitive to small differences in the reaction of the medium on which they were grown. Much work was therefore done upon this point and the optimum reaction was ascertained by experiments with media of varying degrees of alkalinity. It was found that the best results were obtained with a broth medium whose reaction corresponded to + 10 of Eyre's scale, and this has been adhered to in the routine preparation of all brews of vaccine.

In this connection the relative advantage of broth, as compared with a solid medium such as ordinary nutrient agar, was carefully gone into with the result that it was decided to adhere to the use of broth cultures. Vaccines prepared from young cultures grown upon the surface of agar are quite as effective and as readily standardized as vaccines prepared from broth cultures, but the technical difficulties of preparing large quantities of vaccine by the former method were found to be considerable, and further, the dangers of contamination during the process of manufacture appeared greater. Many methods were tested and others devised to obviate these disadvantages, but it was finally decided to rely upon broth cultures. The supposed disadvantage attaching to the use of broth vaccines, in that they involve the injection of a certain amount of peptone, and possibly other poisonous substances, along with the dead bodies of the typhoid bacteria, was not found to have much reality, since comparative inoculations of the same dose of dead bacteria, suspended respectively in saline solution and in the peptone broth in which they had grown, gave similar reactions when tested upon men. At all events, there was no obvious disadvantage attaching to the use of the sterilized broth cultures from the point of view of the local and general reactions occurring in inoculated men.

3. The frequency with which the stock cultures were re-inoculated was also found to be a point of considerable moment in connection with the obtaining of an optimum growth of the strain in a short period. This came out very clearly in connection with

the experimental work at Aldershot where it was found that, as the daily experiments progressed, the vigour of the cultures obtained by daily re-inoculations increased to a very considerable degree. Subsequent experience and experiment have shown that the increased power of growth attributable to this cause has a limit beyond which further increase does not take place, and further, that under some unknown conditions, there may be a later decrease in vigour of growth which is maintained for some time.

4. The degree of aeration has also a marked effect upon the rapidity of multiplication in broth cultures. If oxygen is readily available, growth is much more rapid than where the contrary is the case. This fact was observed in connection with the earlier work carried out at Netley by Sir A. E. Wright and Sir W. Leishman, and further work at a later date has emphasized the importance of securing an abundance of oxygen if good growths are desired. Many technical devices were resorted to to secure this end, without sacrifice of the general principles of simplicity and safety of preparation, and the method which is now in use has been found to answer the purpose satisfactorily. The nutrient broth is sterilized in large flat bottles known as "Roux's flasks." The quantity of broth introduced into each bottle is calculated so as to give a layer of broth of about three-quarters of an inch deep when the flask is laid on its side. After inoculation from the stock culture the flasks are all placed in this position, and the typhoid bacteria, growing in this shallow layer, find no difficulty in obtaining the requisite amount of oxygen and multiply rapidly and satisfactorily.

Without going into further details of such experimental work, it will suffice to say that, by a careful observance of all that has been learnt from these experiments, it is now possible to obtain growths of typhoid bacilli in cultures of 24–48 hours' incubation, equivalent to 1,500 million bacteria to the cubic centimetre, or even more. At times cultures will be found which, in spite of the observance of the usual precautions, fall below the standard, possibly far below it, but it is usually found that there has been some simple explanation of such a result, such as inferiority in the quality of the beef supplied, or irregularities in the temperature of the incubator.

Experiment 20.

Object.—To ascertain the best method of sterilising the vaccine at the temperature of 53° C.

Having decided, for the reasons already stated, to sterilise the cultures of typhoid bacteria by heating them at the lowest temperature that would ensure their destruction within one hour, various experiments were made with a view to selecting the most suitable procedure. It was soon found that the treatment of large quantities of broth vaccine demanded exceptional precautions if complete sterility was to be obtained within this time without allowing the temperature to rise above 53° C. The heat was applied by means of immersing the large flasks in a water bath, which was raised to and maintained at the required temperature. Formerly, large glass jars of a special pattern devised by Sir A. E. Wright were used, which were provided with three side tubes, for the purpose of subsequent mixing of the contents of a number of flasks, and for bottling purposes; over these tubes were fitted short lengths of rubber "pressure-tubing," and the open ends of these rubber tubes were plugged, before sterilisation of the jars, with pieces of solid glass rod. The temperature inside these jars, as has already been mentioned, was gauged by means of the paraffin thermometer devised by Sir A. E. Wright, which was constructed so as to sink when the temperature reached 60° C. At this temperature the contents of these large jars, which had a capacity of 4,000 ccm., were sterilized, as a rule, with certainty in one hour, but when it came to be a question of employing the lower temperature of 53° C., it was found that sterility was not always secured. Further experiments were, therefore, carried out to discover the reason of this failure, as the results had been very uniform when smaller quantities of vaccine were being dealt with. It was found that the weak spot lay in the rubber tubes connected with the glass side tubes. Germs which were lodged within this thick pressure tubing were not exposed to the same temperature as those in the mass of the fluid, and at times such were able to survive the heating for the period of an hour. This point was tested by careful thermometric readings taken inside and outside such tubes placed in a water bath. The jars were therefore discarded and replaced by large flasks of a capacity of 2,000 ccm., with smooth interior and gradually sloping shoulders. The cultures were introduced into these flasks with great care, so as to avoid any splashing of the contents above the highest level at which the whole volume of fluid was to stand. This precaution was necessary to prevent germs drying on the walls of the

flasks, as it is well known that in a dried condition germs will resist a higher temperature. The necks of the flasks were then tightly plugged with sterile cotton wool, and finally covered with a piece of filter paper, which was tied down over them and then painted with melted paraffin. A few perforations were finally made in the paraffined paper, in order to prevent alterations of pressure occurring within the flasks during the heating.

A special pattern of water-bath has been made to receive the flasks and hold them in position buried in the water up to the neck; the bath is heated by gas-burners, and the heat is controlled by a Roux pattern thermo-regulator. In order to ensure the correct temperature being reached and maintained, reliance is not placed entirely upon the thermo-regulator, but, in all cases, a "control" flask, filled with fluid of the same density as the vaccine and treated in precisely similar fashion as regards the plugging and outer cap of paraffined paper, is immersed beside the vaccine flasks. This control flask is provided with a thermometer, penetrating into the centre of the fluid, whose accuracy has been tested by means of a standard instrument, and frequent observations are made of the temperature within this flask throughout the whole of the hour during which the heat is maintained. The whole series of flasks, including the control, are placed in the bath while the water is cold, and the heat is then raised gradually, until the desired temperature of 53° C., is registered by the thermometer within the control flask; the time is then taken, and the heat is kept at this point for one hour.

Samples of the contents of each vaccine flask are taken both before and after heating, the former for the purpose of ascertaining the strength or standardizing, as will be described below, and the latter for the purpose of verifying the sterility. In connection with the latter process it need only be said that the precautions and standards laid down are somewhat more stringent than is usual, both aërobic and anaërobic cultures are made, and are incubated at 37° C. in the usual way, but the period of incubation to which the tubes are submitted is 4 days, and no brew of vaccine is passed as sterile unless it satisfies all requirements in this respect.

Experiment 21.

Object.—To determine the nature and amount of antiseptic to be added to the vaccine after sterilization.

It was decided to continue the use of lysol for this purpose, and the results of various tests have showed that the smallest amount of this antiseptic which can be relied upon to secure sterilization of a vaccine which had been artificially contaminated subsequently to its sterilization by the action of heat, was 0·25 per cent. of the total volume of the vaccine. This amount had been determined by experiments with bottles of old vaccine which had been artificially and grossly contaminated with a mixture of bacteria. The germs introduced into the vaccine consisted both of aërobes and anaërobes, and of spore-bearing organisms as well as vegetative forms. For instance, such mixtures were used as one containing young and vigorous growths of the following bacteria:—*Staphylococcus pyogenes aureus*, *Bacillus subtilis* and *Bacillus tetani*. The contaminated bottles were sealed up in the usual way and left at room-temperature, every day one was opened and a sample taken and examined, both directly and by cultivation, to follow the progress of events. The average of all the experiments done in this way was that it was found that the vaccine once more became sterile within 4 to 5 days after the addition of the greatest quantity and variety of contaminating organisms.

Lysol, in the stronger proportion of 0·4 per cent., is however added now to all vaccine, subsequent to its sterilization by heat, as an additional precaution against accidental contamination if a capsule or phial should be carelessly opened or closed by inexpert hands. If this should be the case, the experiments mentioned above have shown that there can be no danger even if a few germs gained entrance, from the air or elsewhere, since such are bound to be destroyed within a very short time by the action of the antiseptic.

It will be recalled that immunisation experiments upon rabbits (*see* Experiment 12) had shown that it is necessary to allow the vaccine to cool down to room-temperature before adding the antiseptic, otherwise its immunising efficacy is considerably reduced. This is, naturally, always borne in mind in the preparation of the vaccine, and the lysol is never added while the vaccine is hot.

CHAPTER VII.

Standardisation.

Much attention has been devoted to a study of the best means by which the strength of the vaccine at present employed for the immunisation of the troops could be ascertained and uniformity of dosage secured. The great importance of selecting and adhering to a satisfactory method of standardisation was realised and no efforts were spared to attempt a solution of some of the many difficulties which are known to surround this problem. Little help was to be had from the methods in use in connection with some of the other bacterial vaccines employed or, at least, advocated by various continental authorities since the majority of these depend upon measurement of a culture by means of a standard platinum loop which is relied upon to carry, approximately, the same volume of bacterial suspension in all cases. The method of measurement by means of loopfuls appeared to be uncertain and, as a matter of fact, was found by experiment to be subject to much greater variations than appeared desirable.

The task of selecting a suitable method was, to a certain extent, simplified by the decision to adhere to a rigid routine in the method of preparing the vaccine, since whatever method was chosen need only be one which could be relied upon to give accurate information as to the relative strength of consecutive brews of vaccine, prepared in the same manner. The selection or origination of a method capable of giving definite indications as to the relative strengths of vaccines prepared in different ways would have been an infinitely harder task.

In the case, however, of the majority of the experimental vaccines, whose virtues have been tested by the human and animal experiments described in this report, it was possible to employ for standardisation any method which could be relied upon to give a numerical expression to the actual number of typhoid bacilli present in a known volume of the culture or emulsion which was to be converted into a vaccine. As long as this number was ascertained in the first instance the vaccine, however treated, could always be brought up to its original volume and the dose administered could be standardised in terms of the number of bacteria which it actually contained, or which it had originally contained, supposing some or all of the bacteria to have been destroyed subsequently by autolytic action. Similarly, if it were desired only to contrast the strength of one brew of vaccine with another prepared under identical conditions, a method of enumeration of the bacterial bodies would appear to be a suitable one for such a purpose.

While other methods of standardisation have been tried, as will be described below, the majority of the experiments in this province have been directed to attempts to improve existing methods of germ-enumeration or to devise new ones. The method of enumeration most frequently employed in bacteriological work is that of plating out a measured volume of a culture or bacterial suspension, usually highly diluted, upon the surface of a solid medium such as agar, the plates are then incubated and the number of colonies which result is taken as indicating the number of germs originally sown on such a plate. From the volume of fluid originally used and the degree of dilution, which are of course known, it is then simple to determine the number of germs in the original fluid. This method, however, depends for its accuracy and reliability upon several assumptions, such as the following. It assumes that every germ in the original fluid was alive at the time and was capable, when sown upon suitable ground, of giving rise to a colony. Again, it assumes that each germ was isolated from it neighbours, in other words that the suspension was of perfect evenness and that no clumps of several organisms were present. It need hardly be said that such assumptions cannot be made with safety in the case of any culture or emulsion of living bacteria, one can never be certain that all the bacteria are alive, indeed one may be certain that this is not the case, and it is a practical impossibility to secure a suspension of germs of perfect evenness and one which is free from the presence of smaller or larger clumps of bacteria. At the same time, in order to be certain upon these points, many experiments were performed with the result that it was made abundantly clear that the method of "plating out" living typhoid bacteria and enumerating the resulting colonies was quite unreliable for the purpose of accurate comparison of one suspension with another. The results were always underestimated from one or other of the above causes and there did not appear to be any degree of uniformity in regard to the extent of this under-estimation. The method has a certain value for some kinds of experimental work, for instance, it yielded useful results in connection with the estimations of the bactericidal power of the serum of immunised men or animals, as detailed in many of the above experiments, but, when

utilized as a means of controlling the strength and the dose of successive brews of vaccines, the errors were felt to be far too great and the method in general unsuitable for this purpose.

In earlier work there had appeared to be some promise of obtaining a satisfactory method by means of weighing the dried bacterial sediment remaining after centrifugalisation of a given volume of suspension, a system of preliminary trials yielding a numerical coëfficient for the weight of such a bacterial sediment, in terms of the number of germs present in the original volume of fluid. Further experiments with the method had not, however, very satisfactory results, and it, too, was abandoned as being of insufficient accuracy.

Of the other methods which were tried, two more may be mentioned whose object it was to ascertain the bacterial content of the emulsion or broth culture by estimating and recording the degree of its opacity. The first of these was the method which had been devised some years before by Colonel Leishman,[1] and which had been used for the purpose of standardizing a typhoid vaccine. In this procedure the image of a test object was thrown by means of a microscope mirror through a layer of vaccine contained in a cell resting on the stage of the microscope, and the depth of vaccine which was found to be necessary to destroy or blur this image was measured by utilising the coarse adjustment of a microscope to lower into the fluid a piece of plane glass in place of an objective. In this way it was found possible, under standard conditions of illumination, distance of the test object, &c., to determine very small differences of opacity in bacterial suspensions. Careful tests, however, carried out with this method, although it was modified and improved in various ways, did not afford measurements of such small differences in strength as were considered to be of importance from the point of view of the accurate standardization of a series of vaccines.

The second of these methods was an adaptation of the photometer, devised by Major Harrison.[2] In this an attempt was made to measure the degree of interference to the passage of light caused by placing in front of an electric light a flat-sided vessel, of definite thickness, filled with vaccine. The instrument consisted of a long board along which one could slide a carrier holding a paper screen, in the centre of which there was a small grease spot made with a drop of castor oil. At the ends of the board were fixed two Nernst lamps of equal power. When using the apparatus, the point at which the two sides of the screen were equally illuminated (as shown by the disappearance of the image of the grease spot), was noted, three readings being made under the following conditions:—1st, with the light from each lamp unobstructed; 2nd, with a flat-sided vessel full of broth in front of one of the lamps; 3rd, with the same vessel full of the vaccine to be tested, *i.e.*, the same broth but with the typhoid germs in suspension. It was hoped that, in this way, the obstruction to the light caused by the bodies of the bacteria might be measured and a standard fixed with which other brews of vaccine could be compared. The method gave good promise of success at first but was eventually abandoned on account of the fact that every now and then the results showed a considerable error which was attributed partly to differences in the illuminating power of the lamps and partly to the eye-strain which followed on prolonged use of the apparatus.

Another principle of standardization was attempted by estimating the chemical changes which occur in nutrient broth as a consequence of the growth of typhoid bacteria.[3] The principle was to centrifuge a 48 hours' growth of the bacteria until the supernatant fluid was perfectly clear, and then to carry out chemical tests upon the supernatant fluid, contrasting them with similar tests of the broth in which no germs had been cultivated. It was found that there was a rise in specific gravity, in free and albuminoid ammonia and in oxidisable matter as a result of the growth of the bacteria. The total solids were increased but the residue, after incineration, was reduced, as was the amount of chlorine. These changes, however, although marked and constant, were not sufficiently regular for the purpose of forming the basis of a method of standardization, nor were the chemical tests sufficiently delicate to detect such small variations of strength as were felt to be of importance where actual dosage was concerned.

A method of standardization of typhoid vaccine had been suggested by Lamb and Forster,[4] and this was, naturally, given a careful trial. The principle of the method advocated by these authors was the determination of the smallest quantity of vaccine

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[1] *British Medical Journal*, 20th January, 1900.
[2] Journal of the Royal Army Medical Corps, Vol. X., page 583, 1908.
[3] *Idem.*
[4] Scientific Memoirs of the Medical and Sanitary Officers of the Government of India; New Series, No 21, 1906.

which would remove the bactericidal power from a given quantity of normal goat serum when tested upon a measured quantity of living bacteria. The experiments which were undertaken to see whether such a method could be relied upon for the practical estimation of the strength of a series of vaccines were carried out with the "pooled serum" of rabbits, as the goat serum, used by Lamb and Forster, was not available; the principle of the method was not affected by this alteration and all the other details were closely adhered to. The process was found to be a very laborious one, far more so than the blood-counting method described below, but a much more serious objection was found in the fact that it could not be relied upon to detect a variation of ± 25 per cent. in the strength of a vaccine. It was concluded that such a method, with a more satisfactory technique, might prove of great value in instituting comparisons in the value and strength of vaccines prepared under altogether different systems, but that it was unsuitable for the purpose of accurate standardization of different brews of vaccine prepared by the same method.

As none of the above methods had proved of sufficient accuracy, it was decided to experiment with the method of counting the germs devised by Sir A. E. Wright.[9] In this method a known volume of vaccine is added to a known volume of diluted human blood and the two volumes are intimately mixed together. A sample of the mixture is then spread on a slide, stained by an appropriate dye, and the red blood corpuscles and bacteria, respectively, are enumerated in a series of microscopic fields. The normal number of red corpuscles being taken as 5 million per cmm., a figure which in health is very constant, it is a simple matter to deduce the number of bacteria in the volume of the vaccine which had been mixed with the diluted blood.

The above system had been exhaustively tested in connection with the Aldershot work, with a view to the estimation of the strength of the vaccine used in that instance, but the results were found to be somewhat erratic and unreliable. The method, however, had been proved of considerable service in connection with the enumeration of bacterial suspensions other than those of the typhoid bacteria, and it was felt that further investigation might result in improving it and in rendering it a practical method of standardisation for typhoid vaccines. Experiments were therefore undertaken to find out wherein the inaccuracy lay when the method was applied to the enumeration of typhoid germs in suspension. Without going in detail into the various experiments it may be shortly stated that the irregularities were attributed to three main causes:—1st. The difficulties of obtaining a perfectly even film when the mixture of blood and vaccine was spread upon the microscopical slide. Unless such a film is of perfect smoothness, it is inevitable that some parts would contain an undue proportion of either bacteria or blood cells; this would not be a matter of any moment, provided that the relative numbers of these remain constant, but this was by no means found to be the case, and in such unevenly distributed films very different counts were obtained from the thicker and the thinner parts of the film respectively. Every device which had been suggested for the purpose of securing even spreading of the film, and many others, were tested without securing any great increase of accuracy, and even very large counts, carried out upon definite systems of various kinds, were not found to improve things to any marked degree. 2nd. The mechanical action of the various reagents employed for fixing and staining the films in loosening and washing off some of the bacteria from the film. This fallacy it was not easy to prove, but it was felt very strongly by the workers that it was one which had to be taken into consideration. Many fixing agents were employed, and also a variety of stains, in the hopes of finding some method which could be relied upon with confidence to fix in position upon the slide every cell and every germ, but there was no improvement in the results of the counts, as regards greater uniformity, in the case of successive counts of the same vaccine, either by the same observer or by different observers equally practised in the method. 3rd. The bacteriolytic action of the blood fluids upon the typhoid bacteria. This factor was felt to be one which it was impossible to neglect, and, probably, to be that which was responsible for the larger portion of the irregularities encountered. The factor is not one which comes into play in connection with the enumeration of other germs, such as Staphylococci, which are not susceptible to the bacteriolytic action of the blood fluids, but it was found, as indeed was well known before, that the typhoid bacillus was extremely susceptible to such action. Even allowing for the facts that the serum or plasma was diluted to the extent of 1–5 or more in the case of the mixture from which the film was made, and that the period of contact between the blood fluids and the bacteria was short—the latter naturally depending upon the celerity with which the technique could

[9] *Lancet*, 5th July, 1902.

be carried out consistently with accurate work—still, some bacteriolysis was found to take place, and was evidenced by the frequent appearance of pale and swollen bacteria, which could only be stained and recognised as typhoid germs with difficulty. If this effect was produced upon some of the bacteria, as judged by the eye under the microscope, it could not be denied that a similar but more complete bacteriolysis might have overtaken others of the bacteria originally introduced, and that, in their case, the process had gone on so far that they were no longer recognisable. Were this the case, such a film would, when counted, give a result which was an under-estimate of the original bacterial contents of the mixture.

This last factor applies, naturally, with greatest force in the case of such vaccines or bacterial suspensions as are tested before they have been sterilised by heat. Vaccines which have been subjected to the prolonged action of heat, even at such a low temperature as 53° C., are certainly much less liable to this fallacy, since the action of the heat is to make the bacteria far more resistant to bacteriolysis than they were before. An obvious way, therefore, of avoiding this third fallacy would have been to carry out the enumeration by the blood-counting method only after the vaccine had been sterilized by heat, and this was given a thorough trial. Unfortunately, this method was not found applicable, for the reason that one of the actions of the heat to which the bacteria had been subjected was to bring about a small degree of mechanical agglutination by which small clumps of bacteria were formed. This was not very marked, and was sometimes absent altogether, but its occurrence at times made the method inapplicable for general use, such agglutinated vaccines giving readings which were felt to be quite unreliable, and, indeed, were proved to be so when the same vaccine was counted on several occasions. Again, the use of dead bacteria was found to increase the difficulty in securing a perfect mixture of blood cells and the bacteria, probably on account of the fact that since the natural mobility of the bacteria was in abeyance they had a greater tendency to sediment or precipitate than when they were actively mobile.

Such, then, being thought to be the principal factors in the production of the irregularities noted, attempts were made to overcome them by various means. Major Harrison devised a modification,[1] since further amended, of Wright's original technique which, in the hands of practised workers, gives much more reliable results. A brief description of this method may now be given since reliance is, at the present moment, placed upon it for the standardization of the vaccine now being issued for the use of the troops.

A measured volume of the living vaccine is taken, prior to its sterilization by heat, and mixed with a measured volume of diluted human blood in which the blood plasma has been removed from the cells and replaced by an inert fluid which is isotonic and has no bacteriolytic action upon the living bacteria. To achieve this end, a volume of blood is taken from the finger of a normal man and washed into a centrifuge tube with citrate of soda solution. The citrated plasma is pipetted off after all the cells have been driven down by centrifugal action, and is, in its turn, replaced by normal saline solution alone, which is thoroughly mixed with the sedimented red cells.

This is repeated twice, and the alternate washing in saline and centrifuging results in the complete removal of the citrated plasma and its replacement by saline solution, the red cells remaining unaltered and constant in numbers throughout the procedure. After the final washing the corpuscles are once more brought up to their original degree of concentration by means of the marked pipette in which the fresh blood was drawn from the finger. The next stages consist in the intimate mixture of a measured volume of the living culture with a similar volume of a known dilution of the suspension of blood cells in saline solution. The usual dilution found to be convenient for purposes of counting is one part of blood suspension to four parts of saline, but this may be altered as required according as the culture is thicker or thinner than usual. Great care is taken to ensure as thorough admixture of cells and bacteria as possible by frequent drawing in and out of a fine capillary tube, and, when this has been accomplished, a small drop from the centre of the mixture is placed upon a perfectly clean slide, covered at once with a cover-glass and ringed with vaseline. If the strain of typhoid which is employed is one which is actively mobile, a trace of formalin is added before the cover-glass is applied, and this has been found effectual in causing the mobility to diminish to a point at which it no longer interferes with the subsequent enumeration.

At least six preparations are always made and counted in order to control one

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[1] Journal of the Royal Army Medical Corps, Vol. IV., page 313, 1905.

another, and it has been found quite simple to count the unstained bacteria under these circumstances, with a little practice. The specimens are given a little time for the cells to settle before they are submitted to examination. The actual operation of counting is conducted systematically with the aid of a mechanical stage, each field being taken in succession as it comes into view, and the fields to be counted are narrowed to a size suitable for use without causing excessive eye-strain, by means of a counting-disc of fine intersecting glass threads which is dropped into the ocular to rest on the diaphragm. At first, a beginner at this method will underestimate the number of germs considerably—the cells, of course, are easily enumerated—since it is necessary, even with the thinnest possible film, to focus systematically from the under-surface of the cover-glass down to the upper-surface of the slide, in order to make sure that the whole of the bacteria in suspension in the fluid are counted as well as those which, like the red cells themselves, have settled down to the bottom. Practice, however, soon results in a satisfactory degree of uniformity over a number of counts of the same vaccine, and one can rely on keeping the error of a series of such examinations well within the limits of $\pm$ 10 per cent.

This margin of error is smaller than has been been obtained by any other method, and the technique described is felt to be the best at present available for the purpose of standardizing a vaccine by means of enumerating the actual number of bacteria present in a given volume. It may be added, in conclusion, that the uniform character of the reactions obtained in the case of bodies of men inoculated with successive batches of vaccines standardized in the above manner furnishes confirmatory evidence as to the accuracy of the method.

Although the method in question appears to be the best available for the purpose of standardizing the vaccine, as at present prepared, it is hoped that further advances in bacteriological science may afford suggestions which may lead to the elaboration of a more perfect method.

CHAPTER VIII.

Attempts to modify the reactions following Inoculation

This branch of the subject has been studied by direct experiment with vaccines modified in a manner which appeared likely to cause milder reactions, and also by the close examination of the reactions following inoculations with different vaccines, whether devised with the above object or not.

It may be said at once that the latter method has yielded more useful information than the former, and that by taking advantage of all that has been learnt from the careful observation and record of the reactions in groups of men or in individuals, it has been possible to introduce beneficial modification into the procedure of inoculation. The search for a special vaccine which should give little or no reaction, and, at the same time, show no diminution of immunising powers, has not been successful.

The experiments dealing with special vaccines may first be described.

Experiment 22.[1]

Object.—To test on man a vaccine which contained only the bodies of the typhoid bacteria suspended in an inert fluid.

There was considerable ground for thinking that the local if not the constitutional reactions might owe some of their severity to the fact that the peptone and other ingredients contained in the nutrient broth were injected simultaneously with the bacteria, as well as any soluble products derived from the bacteria. Most of the symptoms occurring after a bacterial inoculation are usually attributed to the fact that the bacteria inoculated become dissolved or bacteriolysed in the tissues, and liberate there any endotoxins which they contain; such endotoxins being held responsible for the local pain and swelling, the fever and the other sequela of the inoculation. In order to see whether any reduction of these symptoms could be brought about by inoculating only the bodies of the bacteria and none of the constituents of the broth or the soluble products formed during the growth of the bacteria, an experimental vaccine was prepared in which the broth was removed and replaced by sterile normal saline solution. This

[1] Journal of the Royal Army Medical Corps, Vol. X., page 583, 1908.

was effected in the following manner. A vaccine was made by the customary method by sterilizing a young broth culture at 53° C., and was standardized in the manner just described. After sterility had been verified, the vaccine was subjected to prolonged centrifugalization until the broth in which the bacteria were suspended appeared glass-clear. This clear supernatant fluid was carefully pipetted off and the sedimented bacteria were once more distributed in sterile saline solution. This centrifuging and removal of the clear saline solution was repeated until the bacteria had had three washings in salt solution and could be assumed to be almost entirely free from any traces of the original fluid. The washed bacteria were finally taken up in such a quantity of 0·25 per cent. lysol in normal saline, as would make the emulsion up to the same strength as the original broth culture, viz., 500,000,000 bacteria per cubic centimetre.

A dose of this vaccine was first given to one of the investigators by hypodermic inoculation into the abdominal wall. He noted his sensations very carefully, and concluded that, taken altogether, the local and general reactions were less severe than had followed a dose of standard vaccine taken by him two years before. This difference might, however, have been due to the fact that he was still, to some extent, protected by the previous inoculation, so a further trial was made upon nine young officers who volunteered for the purpose, and the reactions were carefully contrasted with those produced by simultaneous inoculations of the same dose of the standard broth vaccine in a similar group of officers who were being inoculated before proceeding abroad. One had a general impression that the local reaction was somewhat milder in those who had received inoculations of the washed vaccine than in those who had the standard broth vaccine, but there was not a very marked contrast. The constitutional reactions did not appear to differ. All of those who received a first dose of the washed bacteria were given, after 10 days' interval, a second full dose of 1,000,000,000 bacteria of the standard vaccine, and on this occasion, they had a more severe reaction than with the first dose, and also one which was, on the whole, more severe than in the case of the officers whose first injection had been made with the standard vaccine. Whether this was because the first dose had not protected them sufficiently, or whether their reports were biassed by their first experience, it was difficult to determine. Comparative estimations of the anti-substances produced in their blood were made in both groups, but no marked differences were elicited except in the case of the agglutinins, which were very much lower in those who had received the washed vaccine as a first dose, 1—80 as compared with 1—800.

Conclusion.—The diminution of the severity of the reactions consequent on the use of a vaccine consisting of the bodies of the bacilli suspended in an inert fluid was very slight and did not appear to justify the adoption of such a vaccine as a routine procedure.

On another occasion a somewhat similar experiment was made, in this instance with a view to noting the comparative reactions produced by the inoculation on the one hand of the washed bacteria alone and, on the other, of the inoculation of the fluid portion of the vaccine, *i.e.*, the broth in which the bacteria had grown, together with any soluble metabolic products derived from the bacteria. The procedure in this case was to centrifuge a full dose of the vaccine, to remove the supernatant bacteria-free broth and to emulsify the sedimented bacteria with saline solution. These two portions of the original dose were inoculated simultaneously into the opposite flanks of the subject, in this instance the President of the Committee, who carefully noted the local reactions which ensued. He reported that he was unable to detect the slightest difference in the local manifestations. While this experiment confirms the results of the preceding investigation with the washed bacteria it shows, in addition, that there does exist in the fluid portion of the vaccine some substance or substances which are concerned in producing the local symptoms which follow the inoculation of the standard vaccine. The effects of the inoculation of pure nutrient broth, which had not been employed to cultivate typhoid bacilli, had been tested by the investigators on a former occasion and had been found to be exceedingly trivial and transitory.

Another point which would have to be most carefully investigated, if it had been decided to substitute such a washed-bacteria vaccine for that in general use, would be the determination of the respective immunising powers of the bacteria and of the fluid in which they had been grown. That the latter possesses considerable power of inducing the development of the protective substances is undoubted, as was shown by some earlier experiments of Sir A. E. Wright with filtrates of vaccine, prepared from old cultures. As already mentioned, it is still uncertain which of the protective substances is the most

important when an effective and durable immunity has been produced, and even less is known as to the particular portion of the vaccine, for example, the bacteria as compared with the soluble products derived from them, which is of the greatest value in the production of the corresponding anti-bodies in the inoculated organism. In view of this uncertainty, therefore, it might well be that, in removing the fluids in which the bacteria had grown and inoculating nothing but the bodies of the bacilli, one might, unwittingly, be discarding a most essential portion of the vaccine.

Be that as it may, it was at least evident that nothing was to be gained in the way of reducing the reactions following inoculation by employing the bacteria freed from the broth in which they had grown; it was, therefore, decided not to make such a change.

Experiment 23.

Object.—To test the value of the typhoid vaccine advocated by Besredka.

Besredka (Ann. Past. Inst., December, 1902) recommended the use of a vaccine in which a certain amount of an anti-typhoid serum was present, with a view to establishing a more rapid immunity and thus lessening the possible dangers of a negative phase. He claimed for this vaccine that, in addition to the more rapid production of immunity, the local reaction was much less than that which follows the inoculation of bacteria which have not been previously acted upon by an anti-serum. It was, therefore, determined to attempt the preparation of Besredka's vaccine with a view to testing it by the same methods as had been employed in the case of those described above and seeing whether the local and general reactions which it produced were sensibly less than those obtained with the heated vaccine.

The method, although highly praised by French authorities, had been employed to but a small extent upon men and most of the experiments in connection with it had been done upon animals. It was also in the mind of the investigators to see how the method would lend itself to preparation upon a large scale, an essential point in connection with any vaccine which has to be produced in considerable quantities at one time.

In spite of the utmost precautions, it was found that it was difficult to carry out the very complicated and elaborate details of the method with the assurance of sterility, at least when any quantity larger than would suffice for a few inoculations was made, and on this account it was not felt justifiable to institute experiments on man. It was therefore decided to await the further practical test of the method by its inventor, and the publication of results obtained in the actual protection of men against infection before embarking on further experimental work in connection with the preparation of the vaccine. The few results hitherto published on this essential point are in no respect superior to those obtained by vaccines prepared in a manner which is freer from the dangers of contamination.

The addition of anti-typhoid serum to a vaccine, although supported by strong theoretical arguments, loses much of its suggested value in the absence of any definite proof that a negative phase does follow on inoculation, and is sufficiently marked and prolonged to increase the danger of infection in the days following the first dose. No evidence has reached the Committee which convinces them that any such danger exists with the employment of the heated vaccine in the doses and at the intervals which are customary, and, in the absence of such information, there seemed to be little gain in complicating the preparation of the vaccine by the addition of anti-typhoid serum. For these reasons it was agreed that further experiments were unnecessary in this direction at the present moment.

Conclusion.—The advantages of Besredka's method do not appear to outweigh the dangers of contamination to which it is exposed in the process of manufacture.

Better results in the way of reducing the severity of the local and general reactions were obtained in simpler fashion by a careful study of the effects of inoculating the same dose of the heated vaccine under varying conditions and in different sites. Much was learnt in this way, especially in the direction of what to avoid, and every advantage has been taken of accumulated experience in framing the directions for carrying out the inoculations with the vaccine now in use. This experience was gained either by the inoculation of the investigators themselves, or others in close touch with them, or by collecting accurate and full reports as to the effects of the inoculations carried out on bodies of troops.

Before summarising these results and conclusions it must, however, be emphasized that there is unquestionably a great variation in the degree to which different individuals

will react to the same dose, in spite of identity of age, condition, procedure, &c. The explanation of this personal idiosyncracy is far from clear, but its existence is undoubted, and has been noted in connection with the inoculation of vaccines other than typhoid vaccine. Highly strung, neurotic individuals usually have more severe reactions than the average, indeed, men of this type sometimes faint before the needle has touched them; but, on the other hand, strong and well-balanced individuals do not suffer less, on the average, than their weaker brethren. In this connection it may be recorded that the investigators have gathered the impression that an unusually severe reaction indicates an unusual susceptibility to enteric, and they have cases in their memories of some men who suffered such exceptionally violent reactions, especially in the earlier days of inoculation, who subsequently contracted enteric fever; in one case a young medical officer who had such a reaction had two definite and severe attacks of the disease within the following four years. It would appear that, in such a case, there must have been a greatly increased susceptibility to the disease, and that there would be little probability of such an one benefitting to any great extent from inoculation, since his first attack failed to protect him from a second.

An interesting point has recently come to the notice of the investigators in the report of an officer who inoculated a draft of men with a batch of vaccine which had been quite freshly prepared. The local and constitutional symptoms in this case were certainly somewhat more marked than was customary, and it was conjectured, in the absence of any known difference in the strength and constitution of the vaccine, that this might have been due to the fact that it was used so soon after it had been prepared. This appeared to be the case, as inoculations of other batches of men with the same vaccine at a later date gave only mormal reactions. Since then vaccines are not taken into use until they are three weeks old. It is not clear upon what changes in the vaccine this diminution of reaction on keeping depends; it is certainly not progressive, since no noticeable difference can be detected in the effects of the same vaccine inoculated three weeks after its manufacture and when it is three months old—the limit of age which has been adopted.

As regards the manner of the inoculation, this has always been by hypodermic inoculation into the subcutaneous tissue, and no reason has been seen to change this procedure; an experiment was made on one of the workers in which the usual dose of vaccine was inoculated intramuscularly, but this was found to cause more pain than the usual system. On another occasion a dose of vaccine was, unintentionally, injected into the derma, the needle not being thrust through the skin; in this case, as might have been expected, the pain was considerably increased, both at the time of injection and later, when the usual local reaction developed.

One or two instances, which have been reported to the investigators, of exceptionally severe symptoms, following immediately on the inoculation, and in which a condition of temporary collapse appeared to have been induced, may possibly be attributable to the vaccine having been introduced directly into a vein. It is not, however, possible to be certain since no such case has come within their immediate knowledge, but one may assume that, if such an action were to happen, the reaction would be likely to be one of exceptional severity from the sudden throwing of the whole of the dose into the systemic circulation, instead of the gradual process of absorption which probably takes place when it is introduced into the subcutaneous tissues. It may be noted, incidentally, that one of these extremely rare cases was found, on enquiry, to have been inoculated in India with a vaccine which was not prepared at the laboratories of the Royal Army Medical College, and no details are known as to its exact constitution and the amount of the dose. It is hardly necessary to add that careful inspection should be made of the course of the veins in all cases before the needle is thrust into the site selected.

A simple modification, and one which, more than most of the others, has been the cause of reducing the inconvenience of the local reactions, is the change of site of the inoculations. In the earlier days it was the custom to inject the dose into one of the flanks, at a point an inch or two above the crest of the ilium. This site was recommended by Sir A. E. Wright, on the grounds that, since the subcutaneous tissues were exceptionally loose in that region, there was likely to be less pain caused by the subsequent effusion of lymph round the site of inoculation. While this was correct in theory, and confirmed in practice, at the same time the adoption of this site was attended with the disadvantage that, in the event of the reaction being a severe one, the swelling and tenderness in the side made walking difficult or impossible. Many individuals were, therefore, lamed for a day or two, and occasionally rendered quite helpless for a short time. Further, this production of a limp, or at least of a slow and

cautious gait, was not without a more indirect but quite appreciable disadvantage, inasmuch as it was found to exercise a decided deterrent effect upon the men whom it was hoped would be pursuaded to come forward for inoculation upon a subsequent occasion. To such timid spirits the sights of their fellows, apparently crippled by the dose of vaccine was, naturally, far from encouraging.

When the present system of inoculation had been in vogue for a short time, it was soon noticed that the severity of the reactions was more uniformly mild than before, and that the amount of swelling and pain at the site of inoculation were by no means excessive; indeed, in some instances these were practically non-existent. It was felt, therefore, that there was no longer the same necessity to inoculate in the flank. Experiments were made with alternative sites, with the result that it seemed to cause the minimum degree of inconvenience when the dose was given either in the upper arm, at the level of the insertion of the deltoid, or in the infra-clavicular region. Either of these situations permits of the inoculated man being perfectly able to get about without discomfort, and such pain and tenderness as exists is no greater, and certainly of far less duration, than results from smallpox inoculation. The deterrent influence upon others, alluded to above, is naturally far less in question here, as the inoculated individual can get about as well as as anyone else, and, generally speaking, has little inconvenience other than that due to accidental pressure or a jar upon the tender zone surrounding the needle-prick. One or other of these sites is, therefore, recommended on all occasions, in place of the flank.

As regards the general or systemic reaction, a considerable personal variation is evident in this respect; in a large proportion of men, roughly about 25 per cent. of the whole, this is practically non-existent, at least they have no complaints to make on the subject, and their temperatures are either consistently normal or raised ½—1 deg. for a few hours on the evening of the inoculation. On being questioned, such men will say that they felt nothing at all, or at the most, that they felt rather tired and slightly out-of-sorts the next morning. It does not, however, follow, that the absence of general symptoms implies an equal absence of local reaction, as this may be moderately severe, even in the absence of any signs of systemic disturbance. Neither can it be said that any definite correlation has been made out between the relative severity of the two classes of reaction, while it has not infrequently been noted that those who had a severe local reaction with their initial dose, and a slight general reaction, may, on getting their second inoculation, exhibit the converse condition, and *vice versâ*.

It is gratifying to record that of the many thousands of inoculations which have been carried out with typhoid vaccine, only one case of suppuration has been reported at the site of inoculation; in this instance the inoculating officer acknowledged that he had not taken the precautions detailed in the instructions issued with every dose. It may therefore be said that, in this respect, the inoculation of typhoid vaccine beneath the skin is as innocuous as the administration of any hypodermic remedy such as morphia; it is entirely a question of efficient sterilization of the skin and of the syringe, the fluid itself is perfectly sterile, which is more than can be claimed for the majority of hypodermic solutions administered in therapeutics.

Experience has further shown the necessity for taking certain other precautions with regard to inoculation, the neglect of which will almost certainly be followed by a more severe type of reaction than is customary. Of these the following may be mentioned.

1. The time of day at which a man is inoculated.—While this is a point of little moment in the case of those whose time is their own, and who can, if necessary, rest or go to bed if they feel inclined, it is otherwise with the soldier, who, if feeling the effects of the vaccine sufficiently to make him wish to lie down and be quiet, has no other refuge than his crowded and noisy barrack room. Bearing this in mind, it is always recommended to those whose duty it is to inoculate large or small batches of soldiers to arrange for the carrying out of the inoculations in the afternoon, and not in the morning. By doing so it follows that at the time at which the symptoms, both local and general, may be expected to reach their maximum degree of severity, namely, four to six hours after the inoculation, it will be about the customary time for the men to go to bed, which is certainly the best place for them if they are feverish or suffering pain at the site of inoculation.

2. Avoidance of undue muscular fatigue.—Warning is always given on this point, as the result of the experience gained in the case of those who have not taken this precaution. In many, indeed it may be said in most, cases the individual has no symptoms of any sort for some three or four hours after he has been inoculated.

Feeling, therefore, as he does, perfectly well, he is apt, unless cautioned, to do anything which he is accustomed to do at this time; for instance, he may join in a game of some sort such as football. The result of this is almost always the aggravation of the symptoms, both local and general, sometimes to a considerable degree. There is no reason to forbid moderate exercise, such as walking, but anything more violent should be avoided.

3. Exposure to the sun, especially if in the tropics.—This, like violent exertion, must be avoided, as it has been known to cause a tendency to syncope. In the case, therefore, of the inoculation of a body of men in a tropical station, it is always best to arrange to have this carried out in the men's own barrack rooms and not in the hospital, especially where the latter procedure entails a long walk back to barracks under a sun which, even in the evening hours, is not to be despised.

4. The avoidance of alcohol.—This, the last of these small precautionary measures which will be mentioned, is one of the most important. It is common and, indeed, almost invariable that the drinking of alcohol in any form within the 24 hours following on inoculation will be followed, sometimes almost instantaneously, by a marked exacerbation of the local reaction. This is frequently noted in spite of the invariable warning given by the inoculating officer, for, like most good advice, it is often neglected. Those who neglect to practice this abstinence for the few hours necessary almost invariably pay the penalty in the shape of acute suffering at the site of inoculation. The nature of the alcoholic drink and its strength do not appear to matter as the same result is obtained even after the drinking of a glass of beer or a weak whisky and soda.

In this connection Sir Wm. Leishman states that it has several times been reported to him by junior officers who have carried out the inoculations of large numbers of men that the severity of the reactions had a distinct and direct relationship to the habits of the men concerned; those who were teetotalers had extremely mild reactions, while the regular beer drinkers, even such as were quite moderate in this respect, had reactions of a distinctly more severe type.

The observance of the above simple precautions has had a distinctly beneficial effect in lessening the severity of the reactions, and it is now quite the exception to encounter the severe types which were not uncommon in the earlier days. In nine cases out of ten the symptoms, both general and local, are trivial and in 24—48 hours the inoculated man is quite himself again, except perhaps for a slight tenderness at the site of inoculation which may last for a day or two longer. Arrangements are always made for the men to be excused by their Commanding Officer from drills and heavy fatigues for one or two days after inoculation and it is very exceptional for a man to be incapable of resuming his full work after 48 hours.

Although, as stated above, severe reactions are rarely seen with the present vaccine, still the local reaction is moderately severe and the general reaction is sufficiently uncomfortable. It does not seem possible at the present moment to abolish these completely without at the same time diminishing or destroying the immunising properties of the vaccine. It is possible that future advances in connection with the study of immunity may render this practicable, but in the meantime a certain amount of discomfort appears inevitable. This being so, it might be thought that these reactions would constitute a grave barrier to the voluntary inoculation of soldiers, but such is not the case. What matters far more than this is the manner in which the case for inoculation is presented to the soldier; if this is done by one who has the facts in his possession and, still more important, by one who is himself convinced of its benefits, it is surprising how large a percentage of the men come forward and how very little they think of the transitory inconvenience caused by the reactions.

CHAPTER IX.

Attempted Immunisation by Ingestion.

It was thought possible that ingestion might produce immunity by the vaccine being absorbed through some portion of the gastro-intestinal tract, since some recent experimental work had had encouraging results. Although the majority of the experiments in question were performed in connection with tubercle, and with anti-serums in place of bacterial vaccines, it seemed probable that if the dead bacteria could be conducted safely past the stomach and escape the destructive action of the gastric juice, they might be absorbed through the intestinal walls and might then be able to give rise

to an efficient production of the specific anti-bodies, just as when the same bacteria are introduced below the skin by means of a hypodermic syringe.

Although the general results of the experiments with typhoid vaccine detailed below did not result in demonstrating ingestion to be as effective as the method in current use, still the results to a certain extent justified expectation and are of considerable scientific interest. It is quite possible that here, too, further light may be obtained from analogous experiments in connection with other diseases which may lead to the elaboration, at a later date, of a safe and reliable method of immunising by the mouth instead of by inoculation.

Experiment 24.[1]

Object.—Attempted immunisation by ingestion of a vaccine which had been killed by glycerine.[2]

The first experiment consisted in the swallowing of a suspension of typhoid bacteria which had been killed by the action of glycerine. An emulsion was made in sterile neutral glycerine of a mixture of two strains of typhoid bacteria, one non-virulent the other virulent. These strains were grown on the surface of agar for 24 hours, and the glycerine was added in the proportion of 1 ccm. to each tube. The suspension was found to be sterile at the end of 24 hours, and as soon as this fact had been verified doses were swallowed, commencing with 0·1 ccm., and increasing to 0·75 ccm., at first daily, and later every two or three days, until in all 4·5 ccm. had been taken—the equivalent of the growth from 4½ agar tubes. The doses were swallowed as far as possible on an empty stomach. Beyond the apparent production on one occasion of very slight mental confusion, no symptoms were noted. The subject of this experiment (W.S.H.) was not normal, having been inoculated by the ordinary method a year previously.

Tests were made in connection with the phagocytic ratio, for agglutinins and for bactericidal substances. As regards the first, the phagocytic ratio, this was found after 10 days to have risen to 2·4, and it was maintained at this level for 8 days, but it did not subsequently exceed the normal, although three further doses, amounting to 1·9 ccm., were swallowed. There was a rise in the bactericidal power of the serum up to a maximum of 1–110, as compared with a normal value of 1–10, tested under the same conditions. This high bactericidal level was attained 52 days after the commencement of the treatment, and 2 months later it still stood as high as 1–80. There was no rise in the amount of agglutinins, which remained at the same height as at the beginning of the experiment. In connection with the last point an experiment was performed upon a guinea-pig in order to see whether there had been any destruction of the agglutinogens by the glycerine. This guinea-pig, however, responded well to a hypodermic dose of the glycerine vaccine, and in 17 days its serum agglutinated up to 1–100. It was concluded, therefore, that the absence of response in the case of the ingestion experiment was due to the method of administration.

Conclusion.—The swallowing of vaccine killed by glycerine, in the case of a previously inoculated man, resulted in a marked rise in the bactericidal power of the serum, and in the substances concerned in phagocytosis, although no rise occurred in the case of the agglutinins. The ingestion of this vaccine was not followed by any untoward or unpleasant symptoms.

Experiment 25.[3]

Object.—Attempted immunisation of a normal man by means of the ingestion of a vaccine killed by glycerine.

The former subject having previously been immunised by inoculation of typhoid vaccine, the experiment was repeated upon another officer, who had not been inoculated, and who had never suffered from any continued fever resembling typhoid. The serum of this officer had previously served as a control normal blood on many occasions, and showed a normal phagocytic ratio, no agglutinins, and a bactericidal power of 1–10.

The vaccine used was the same as in the former experiment, and was given by the mouth in gradually increasing doses, commencing from 0·05 ccm. and rising to

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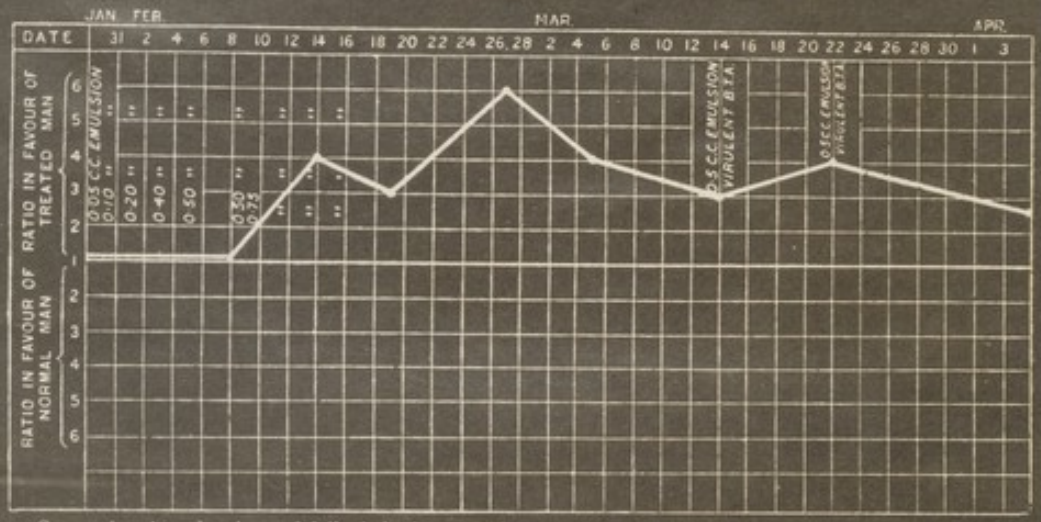
[1] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907; Vol. X., page 583, 1908; and Vol. XI., page 327, 1908.

[2] The experiments in connection with immunisation by the mouth were conceived and carried out mainly by Major W. S. Harrison, who devoted special attention to this subject.

[3] Journal of the Royal Army Medical Corps, Vol. VIII., page 472, 1907.

0·75 ccm. There were no symptoms except on one occasion, when there was some gastric disturbance, which was probably due to another cause.

CHART XIX.



Curve showing the rise and fall of the bactericidal action of the serum of a normal man (T. H. G.) after swallowing a glycerine emulsion of *B. typhosus*, the grouped sera of two normal men being taken as unity.

The bactericidal power was markedly raised by the treatment, reaching a height of 1–60 one month after it had commenced, and at the end was still at a level of 1–25, as compared with a normal of 1–10. (*See* Chart XIX.) There was again very little evidence of appearance of agglutinins, which reached on one occasion a maximum of 1–30, and at the end were incomplete in 1–20. The phagocytic ratio, on the other hand, was depressed during the whole of the experiment, and for 19 days after its termination, when it began to rise, and finally stood somewhat higher than the normal. In this respect the behaviour of this officer's serum was in marked contrast to that of the previously immunised man, in whose case it will be recalled that these substances exhibited a most striking and well maintained rise.

Conclusion.—The administration of a glycerine-killed vaccine by the mouth, in the case of a normal man, resulted in the elevation of the bactericidal power, and, in a slight degree, of the agglutininating power, but the substances influencing phagocytosis were depressed throughout the treatment, and appeared to indicate a true negative phase.

The two experiments recorded above, although, of course, far from conclusive in many points, at least served to show that the administration of dead typhoid bacteria by the mouth was able to induce marked modifications in the amounts of the various protective substances, and, to this extent, they were decidedly encouraging. The experiments were, therefore, resumed on a subsequent occasion, and with a different procedure.

Experiment 26.[1]

Object.—To ascertain the effect of swallowing dead typhoid bacteria suspended in fat.

In this instance the bacteria were grown on agar, emulsified in sterile water, and killed by desiccation over sulphuric acid. The resulting mass was found to be sterile in 48 hours, and was then finely ground in a mortar and mixed with lard, to which a little stearine had been added to make a firmer mass. The admixture with fat was made in the hope that in this medium the bacteria would be enabled to pass undigested through the stomach, and would subsequently be absorbed in the small intestine with the fine particles of fat. The mixture was divided into pills, each of which contained the equivalent of one agar tube of culture.

The pills were swallowed at short intervals by W.S.H., the subject of the first ingestion experiment with glycerine-killed vaccine. They were taken at intervals of 2 days, half an hour before breakfast, and caused no unpleasant symptoms, except for slight nausea on one or two occasions, which was attributed to the taste of the stearine.

[1] Journal of the Royal Army Medical Corps, Vol. X., page 583, 1908.

The various protective substances in this instance already stood considerably above normal levels on the experiment being commenced, but the effect of the ingestion of the pills was evident in the production of a rise in the amount of the agglutinins, and in the substances concerned in phagocytosis. On the other hand, the bactericidal power of the serum was markedly depressed by the treatment, and only rose to and above the normal level on the pills being discontinued.

Conclusion.—The ingestion of pills containing dead typhoid bacteria in fat, in the case of a man already immunised, was followed by a response in the production of certain antibodies; the bactericidal power was, however, lowered, possibly on account of over-dosing.

Experiment 27.[1]

Object.—The ingestion of dead typhoid bacteria suspended in lard and enclosed in gelatine capsules.

In this experiment a normal man was the subject, he had neither been inoculated nor had enteric fever. In the three previous experiments the doses were large, and had been given in rapid succession, the purpose being the double one of ascertaining whether any disagreeable constitutional effects were produced, and whether it was possible to induce modifications in the quantities of the various protective substances. In the light of these experiments, a somewhat different procedure was adopted in this instance. The doses were given at lengthy intervals, about one month between each, and the estimations of the various substances were carried out at intervals during the intervening periods. The purpose here was to ascertain more accurately the effects of each individual dose in the case of a perfectly normal individual.

In this instance no agglutinins appeared at any time, although towards the end of the experiment the doses were taken at shorter intervals of 10 days. The bactericidal power also was not affected in any way. A different result was, however, obtained in the case of the substances influencing phagocytosis. In the case of the phagocytic ratio, there was a distinct rise 13 days after the first capsule had been taken, and this rise was repeated, though at shorter intervals, on each subsequent occasion on which a dose was taken. The levels attained were not very high, except upon one or two occasions, but they were consistently higher than normal, with the exception of one record, which was subnormal, but this was probably connected with the fact that the subject was at this time suffering from a severe coryza.

Conclusion.—Single doses of dead typhoid bacteria, suspended in lard and enclosed in gelatine capsules, when taken at long intervals, were followed by a rise in the substances concerned in phagocytosis, but none in the case of the agglutinins, nor was any effect produced upon the bactericidal power of the serum.

Experiment 28.[2]

Object.—The ingestion of dead typhoid bacteria suspended in fat and enclosed in gelatine capsules.

In this experiment, which was done some months later than those recorded above, three officers and one private of the R.A.M.C. were the subjects; two of the officers had been previously inoculated with typhoid vaccine, the other officer and the private had not, and none had suffered from enteric fever. These four volunteers swallowed the capsules of typhoid bacteria at different intervals and in different numbers, the details of which are fully recorded (*loc. cit.*). The majority of the capsules contained bacteria which had been killed by exposure to the vapour of chloroform, but in a few instances they had been killed by heating in the fat to a temperature of 60° C. Each capsule contained the equivalent of one-half of the growth on the surface of an ordinary agar tube, incubated for 24 hours.

The tests which were carried out were estimations of the opsonins by both Wright's and Klien's methods, and the estimation of the agglutinins. No analyses were, on this occasion, made as to the bactericidal substances. At each separate estimation a control observation was made upon the serum of a healthy and non-immunised man. The results in the case of each subject may be considered separately.

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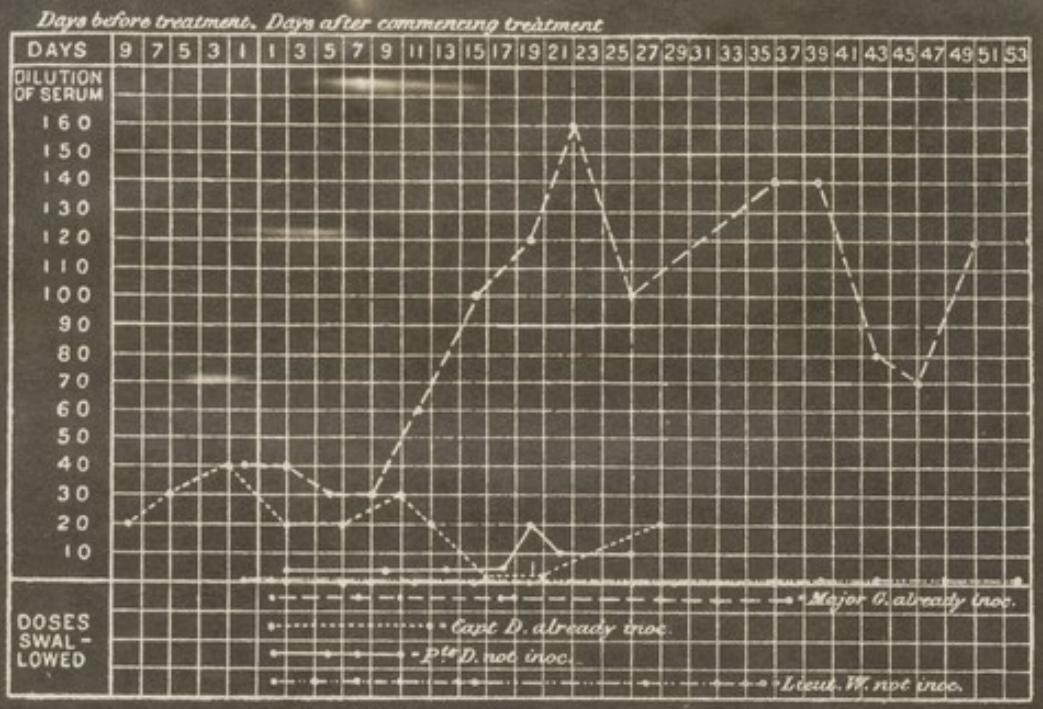
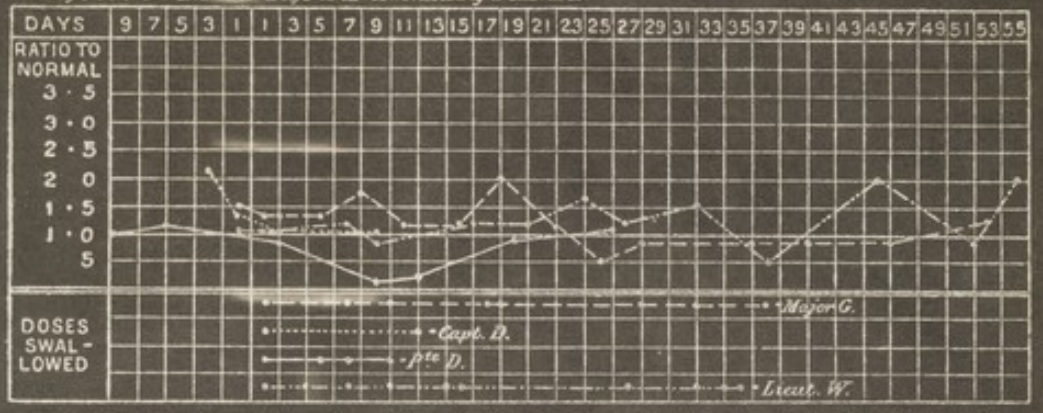
[1] Journal of the Royal Army Medical Corps, Vol. X., page 583, 1908.
[2] *Idem*, Vol. XI., page 327, 1908.

CHART XX.



Ingestion of dead typhoid bacteria. Curves of *agglutinins.*

CHART XXI.



Ingestion of dead typhoid bacteria. Phagocytic indices of four volunteers, tested by *Wright's method.*

1. Major G. had been previously inoculated with the usual vaccine two years before. He took in all eight capsules within a period of five weeks. The treatment was followed by a marked and well-maintained elevation of the phagocytic power as tested by Klien's method, but, on the other hand, Wright's method did not show the same result, the readings falling below the normal after the fifth dose had been taken, and not recovering until a fortnight after the last dose. Agglutinins which, at the beginning of the experiment, had stood at a level of 1—40, rose to 1—160, and at the conclusion of the experiment still remained as high as 1—120.

2. Captain D., previously inoculated some years before. Only two capsules were swallowed in this case, as the subject was upset by the treatment, considerable digestive disturbance ensuing. His phagocytic ratio which, at the beginning of the experiment, stood well above normal, fell, after some oscillations, to within normal limits. Agglutinins, which stood at 40 at the beginning, fell to vanishing point while the experiment was in progress, but subsequently recovered on the capsules being discontinued.

3. Lieutenant W. had neither been inoculated nor had enteric fever. He took 10 capsules over a period of one month. In his case they gave rise to no unpleasant symptoms, but his blood, throughout the whole of the experiment, showed no signs of the development of either increased phagocytic power or of agglutinins. It may also be

mentioned that this officer, when inoculated at a later date with the ordinary vaccine, had an unusually severe type of local reaction, and his general reaction, although not exceptionally severe, was prolonged into the second day. It appears possible that in his case the large number of capsules taken, equal to five agar tubes of culture, had induced a certain measure of hyper-sensibility to the subsequent hypodermic inoculation.

4. Private D. had neither been inoculated nor had enteric fever. He took four capsules within 10 days. A transient rise of phagocytic activity was evidenced by Klien's but not by Wright's method, but this soon disappeared, and his phagocytic power remained normal after the termination of the experiment. A trace of agglutination appeared in his serum eight days after the last dose had been taken. He was not upset in any way by the ingestion of the capsules. (*See* Charts XX. and XXI.)

Taking these four experiments as as whole, and bearing in mind the results of those recorded in the foregoing series, it cannot be said that they demonstrate ingestion to be an efficient substitute for inoculation. It is obvious that the ingestion of dead typhoid bacteria is capable, under certain conditions, of increasing the amount of typhoid protective substances in the blood, but the results which have been obtained by these experiments have been too irregular and apparently too little under control to warrant the application of the method upon a larger scale, at all events for the present. There appear to be great individual variations in the manner in which different persons react to the ingested bacteria and some of the cases suggest that the immediate effect of the treatment had been the production rather of an increased susceptibility than an increased protection. The reason for this variability is far from clear, but it is obvious that the conditions of the intestinal tracts of different individuals must differ widely as regards secretions, bacterial flora, absorptive capacity, &c., and it is probable that in some instances many bacteria may have been absorbed into the system, in others few, or possibly none. It is possible that further experience may indicate a line of procedure from which more regular results might be anticipated; should this occur it is proposed to resume these experiments at a later date. The whole question of the absorption of germs and their products through the intestinal walls is one which is still in its infancy, and the valuable results which have followed the deliberate modification of the bacterial contents of the intestine by the swallowing of cultures of lactic acid bacilli suggest possibilities which may eventually render intestinal immunisation with typhoid bacteria practicable.

Conclusion.—The results obtained by the ingestion of pills or capsules of dead typhoid bacteria are too irregular and too little under control to justify the adoption of this method of immunisation, at all events, at the present moment.

CHAPTER X.

Miscellaneous Experiments.

A.—*The influence of the virulence of the strain upon the immunising properties of a typhoid vaccine.*

The question of the virulence of the strain which should be used for employment in a bacterial vaccine is one which has given rise to considerable differences of opinion among bacteriologists, some holding that a non-virulent strain should be selected and others that the best results were only to be expected when a strain of high virulence was employed. Since the introduction of typhoid vaccine into the Army, none but non-virulent strains have been used, as Sir A. E. Wright was of the opinion that such presented definite advantages over virulent strains. It was felt, however, by the Committee that experiments should be instituted to test their relative merits by the immunisation of animals and a study of the manner in which their blood serum reacted to virulent and non-virulent vaccines respectively.

It was also realised that, unless very definite information was obtained as to the inferiority of the non-virulent vaccine, it would not be advisable to substitute for it a virulent one on account of the more severe nature of the reactions which follow inoculations of a virulent vaccine, and the adverse effects which this would be likely to have upon the number of men volunteering for inoculation.

Reference may first be made to the following tests which were conducted with a view to ascertaining in how far the serum of a man who had been inoculated with the usual non-virulent vaccine was capable of reacting with other and more virulent strains.

Experiment 29.[1]

Object.—To ascertain the action of the serum after inoculation upon strains of typhoid bacteria other than that which had been employed in immunisation.

The serum used was that of an officer who had been inoculated some months before with a vaccine prepared from the non-virulent strain, "R," at that time in general use, and who had, on a subsequent occasion, ingested pills and capsules of dead bacilli of the same strain. The blood serum of this officer was tested as regards its bactericidal power against three different strains of typhoid bacteria. 1st. The non-virulent strain which was employed in the preparation of the vaccines with which he had been immunised. 2nd. Another strain of equally low virulence. 3rd. A more recently isolated strain, which possessed a considerable degree of virulence for guinea-pigs. The bactericidal power of a sample of his serum was determined against each of these three strains, the principle of the tests being the admixture of identical test doses of each strain with a series of dilutions of the serum, the incubation of these mixtures at blood heat for one hour, and the determination of the bactericidal power of each dilution of the serum by plating the mixture out on McConkey's mannite-bile-salt medium.

The results showed that the bactericidal power of the serum was equally high with each strain, as contrasted with the bactericidal power of a normal control serum, in the case of each variety of bacillus and of each dilution of serum. This was in accord with the results of similar experiments, which had been made at an earlier date, and although the immediate object of the above experiment was to elicit information bearing upon the comparative values of monovalent and polyvalent vaccines, it also bears upon the question at issue of the advantages or disadvantages of a virulent *versus* a non-virulent vaccine.

Conclusion.—The serum of a man inoculated with a non-virulent strain contains substances which are effectively bactericidal, not only against the strain used to vaccinate him, but also against other and more virulent strains.

From the above experiments, and others of similar nature, it appeared certain that a vaccine prepared from a non-virulent strain might be trusted to produce substances which would act effectively upon the virulent strains to which the individual might subsequently be exposed, but this did not settle the question of the possible superiority of a virulent vaccine in the way of producing a larger amount of protective substance than would result from the use of a non-virulent strain. The following experiments were, therefore, carried out to see whether animal experiments would throw light upon the subject.

Both of the immunisation experiments upon animals, in which this point was dealt with, either directly or indirectly, have already been detailed (*see* experiments 13 and 17), so it will suffice briefly to recall their nature and conclusions. In experiment 13, two vaccines, which had been killed by the action of glycerine, were used to immunise groups of rabbits; one of these vaccines had been made from a non-virulent strain, the other from a virulent one. The conclusions reached from this experiment, in so far as the factor of virulence was concerned, showed the advantage to incline to the side of the vaccine which had been prepared from the virulent strain; the differences, however, were not striking, and, such as they were, appeared more in evidence in connection with the bactericidal power than in the case of the agglutinins or of the substances concerned in phagocytosis.

Experiment 17, which was carried out at a later date, was directly concerned with the experimental testing of this question of virulence, and, in this instance, the vaccines whose immunising power were contrasted, were sterilized by heat at 53° C. in the manner which is now employed for the vaccine issued for the use of the troops. The conclusion reached in this experiment was that as satisfactory a development of protective substances was induced in the blood of inoculated animals by the use of a non-virulent heated vaccine as by employing one which had been prepared from a highly virulent strain.

B.—*The influence of typhoid vaccine on paratyphoid infections.*

The above question has come to be one of considerable importance in connection with anti-typhoid vaccination for two reasons, first, because the more frequent employment of modern methods of blood-culture in the Army has demonstrated that paratyphoid infections are far from uncommon in some tropical stations, and second, because an

[1] Journal of the Royal Army Medical Corps, Vol. X., page 583, 1908.

appreciable number of cases of continued fever which have been subsequently proved to be paratyphoid in nature have occurred in men immunised against typhoid and have been recorded as instances of the failure of typhoid vaccine. The following experiment was therefore undertaken :—

Experiment 30.[1]

Object.—To ascertain whether the serum of men immunised with typhoid vaccine exerted any power upon paratyphoid bacteria.

The sera of two men who had previously been inoculated with typhoid vaccine was tested as to their agglutinative, bactericidal and phagocytic power against Paratyphoid "A" and Paratyphoid "B," respectively. The results were contrasted with those obtained in the case of the blood of a normal man who had not been inoculated and who had never suffered from any continued fever resembling typhoid or paratyphoid. The results were as follows. Bactericidal substances for paratyphoid bacteria were present in the serum of the inoculated men, but in no greater amount than in the blood of the normal uninoculated man. The variety "A" was more readily killed by the diluted serum than the variety "B," but in neither case could any distinction be found between the immune blood and the normal. As regards agglutinins, a slight "group agglutination" was found with both men, and in the case of each variety of paratyphoid bacilli, the highest dilution giving the reaction being 1—30; the normal serum, on the other hand, showed no trace of agglutinating power on the paratyphoid bacilli even in a dilution of 1—10. The phagocytic power of the inoculated men, when tested upon the two varieties of paratyphoid, showed no distinct superiority to that possessed by the blood of the normal man. At the time of the tests the phagocytic power of the inoculated men tested upon typhoid bacteria was twice as great as that of the non-inoculated control.

Conclusion.—Inoculation with typhoid vaccine does not appear to confer any appreciable degree of immunity against subsequent infection by paratyphoid bacteria.

Since the above experiment was made, the number of cases of paratyphoid infection which have been brought to light by means of modern methods of blood examination has continued to increase. Although the disease is not quite so severe as true typhoid fever and has a lower mortality, at the same time it gives rise to a considerable amount of sickness, and experiments are now in progress with a view to determining whether it would be possible to utilise a vaccine combining typhoid and paratyphoid bacteria which would be protective against both diseases, without bringing about any loss of efficiency in the typhoid portion. These experiments are not sufficiently advanced to allow of their inclusion in this report, but the results so far are favourable to the idea that such a vaccine may be prepared with good prospect of its exercising a double protective action against the two diseases.

C.—*The employment of living bacteria for immunisation.*

No experiments have been performed with living bacteria, in all instances the typhoid bacteria employed in the above described experiments had been killed by one or other of the methods detailed. At the same time there is now considerable evidence in connection with other bacterial diseases, notably in the case of cholera, that the inoculation of the living germs is an effective and reliable method of protection against the particular disease. The advocates of these living vaccines claim for them the production of a more rapid and more durable immunity than results from the use of dead germs, and, in some cases, that the degree of immunity which is produced is also higher. Apart from smallpox vaccine and Pasteur's anti-rabic method, both living vaccines, the cholera vaccine of Haffkine and the plague vaccine of Strong, may be quoted as recent examples; both of these living vaccines have been given by hypodermic inoculation with good results and without reproducing the disease which they were designed to prevent. A living typhoid vaccine has not infrequently been advocated, and has recently been used by Castellani, it is said with good results, but the Committee have felt that the inoculation of living typhoid germs was undesirable in the case of the Army for reasons which will occur to everyone, not the least of these being the fact that such inoculations could only be carried out by trained bacteriologists at central laboratories, a procedure which would greatly narrow the utility and applicability of the

[1] Journal of the Royal Army Medical Corps, Vol. X., page 583, 1908.

process. Should further experience with living typhoid vaccines prove that such actually possess a much higher immunising efficiency than dead cultures, it might be advisable to reopen the question, and to investigate the method with a view to its adaptation on a large scale to the inoculation of the troops, but such a superiority has not yet been demonstrated, and the arguments which are used in support of the method are chiefly concerned with animal experiments and the blood changes produced in them and in men; statistical evidence of actual protection against enteric fever is still lacking.

CHAPTER XI.

The Blood Changes resulting from Typhoid Fever.

The following investigation was initiated with a view to determining the fluctuations of the various protective substances in the blood of men suffering from or recently convalescent from typhoid fever. It was felt that a series of observations on such cases might throw a valuable light upon the degree of importance attaching to the various protective substances, and thus help towards the recognition of that one of them which might be taken as the best indicator of the degree of immunity, whether such immunity be the natural immunity acquired by the convalescent or the artificial immunity following on the inoculation of typhoid vaccine.

The blood changes in question have already been investigated by various bacteriologists, but the results obtained proved of little assistance in connection with the question of typhoid vaccine, owing to the fact that the qualitative and quantitative methods employed varied widely from those which have been used throughout the foregoing series of analyses. It was, therefore, difficult to contrast any two series of experiments and, the need for some such standard of comparison between the convalescent and the inoculated man being felt to be urgent, the following investigation was undertaken as time and opportunity were forthcoming.

Since it was not possible for the investigators to study active cases personally, nor to follow up individual patients during the months succeeding an attack of typhoid, they endeavoured to secure the information of which they stood in need in the following manner. The sample of blood serum obtained from each patient, or convalescent, on being received at the laboratory, was tested at once, and once only, for agglutinins, bactericidal substances and opsonins, the methods employed being those which were utilised in the case of the animal and human experiments just recorded. These tests were carried out as material offered, and 41 cases were dealt with within 6 months. The majority of these men were convalescents admitted to the military hospitals of Netley and Millbank, while a few cases of active enteric fever, which occurred during this time, were also utilised. Uniformity was to a certain extent secured by arranging that the blood serum should, in all cases, remain in contact with the clot for 24 hours before it was drawn off and submitted to analysis.

A chart was drawn up in connection with each substance on which the observation made from the particular serum and recorded under the particular week dating from the commencement of the original attack. It was hoped that, in this way, the mean value of each substance might be ascertained during each week of the disease and each week in the months immediately following convalescence, and that curves of those means might subsequently be constructed which would yield useful information. As was anticipated, considerable individual variations were encountered and it was therefore decided, when it came to be question of drawing the curves from the collected observations, to neglect an isolated record standing under any one week and to mark a point on the curve of the averages only when this could be arrived at from the mean of two or more tests, on separate individuals, made during the same week of the disease or of convalescence. In the later months of convalescence, however, when such individual variations should be less in evidence, the mean of two or more observations occurring within the same fortnight was admitted, supposing there was no more than one observation in a given week.

Information was also elicited in connection with each case as to whether the original attack had been mild, ordinary or severe, but it was found that the number collected contained so great an excess of severe cases that little of value could be anticipated from this side. The preponderance of these severe cases was due to the fact that most were soldiers who had been invalided from India or elsewhere abroad on account of the

severity of their attacks or their protracted convalescence; mild cases of typhoid fever are no longer sent home, but undergo their convalescence at a sanatorium or hill station. The few mild or ordinary cases which were tested were either local cases or soldiers who had been invalided for some other disease.

The details of this lengthy experiment have already been published, together with charts of the curves, and their general character only will be indicated.[1] The cases ranged from the 2nd week of the disease up to the 7th month of convalescence, but were principally grouped round the 3rd and the 4th months from the commencement of the attack; the cases tested during the actual attack and immediately after this were comparatively few. To deal with the various substances in turn.

1. Agglutinins. These have already formed the subject of many and exhaustive investigations and no fresh facts were elicited, the results being quite in accord with what has been noted by other observers. From a mean level of 1—250 during the 2nd week of the disease the agglutinins fell rapidly after the decline of the fever and remained, with rare exceptions, at a low level, between 1—100 and 1—50, for the succeeding months; in no case was a negative result recorded, even 8 months after the attack. Considerable individual fluctuations were naturally in evidence and one case of exceptionally high agglutination was noted during the 25th week, 1—1000, it is possible that this case might have been a "typhoid carrier," but there was no opportunity of putting this to the test. The only case which terminated fatally, subsequent to the test of his serum, showed, in the 5th week of the attack, a comparatively low agglutination value, 1—50.

2. Bactericidal substances. The technique employed in the animal experiments was adhered to in the measurement of these substances and each observation was contrasted with a similar one carried out on the blood of a normal man. The curve plotted from the means of the observations under each week shows a rapid decline of these substances after defervescence and a fall to or even below the normal level 6 months after the attack. The average value in the cases which had been classed as "mild" was considerably higher than that of those classed as "severe," the inequality of the groups must, however, be borne in mind in this instance. The fatal case, when tested, showed a comparatively high bactericidal power, a point which will be referred to again.

3. Phagocytic ratio. This was tested on each occasion by Wright's method, heated serum only being employed, and each observation was controlled by a similar one carried out on the serum of a normal man. Individual variations were again marked, but the curve plotted from the means was interesting and showed a marked contrast to the curve of the bactericidal substances. Beginning at a low point, little above the normal, the phagocytic power gradually rose in the weeks following convalescence, and this high level was well maintained and showed little tendency to diminish even in the 8th month, when the bactericidal power had fallen to or below the normal. The average values in the mild cases were slightly higher than in the severe ones. In the fatal case the interesting observation was made that the phagocytic power was below normal in the 5th week of the disease, this being the only instance in which a sub-normal reading was recorded during the first 3 months.

Too much stress is not laid upon the results of this investigation, the numbers being comparatively small, and the individual variations considerable, but, at the same time, some general conclusions appear justifiable. The steady and progressive decline in the agglutination curve, and, still more, in the bactericidal curve, stood out in striking contrast to the curves of the phagocytic substances which tended to rise as convalescence became more established, and were still high at a time when the bactericidal power of the blood had fallen to or below normal. If, therefore, it is the case that the bactericidal substances tend to diminish after an attack of enteric while the substances concerned in phagocytosis tend to increase, it appears reasonable to attribute to the latter a more important rôle in the protection of the individual against a subsequent attack. It is true that the agglutinins, in spite of the rapid initial fall, are frequently found to be demonstrable several years after an attack, but there is little tendency at the present day to attribute to these bodies any important part in the defensive organization. In the view of Sir William Leishman, and his fellow-workers, estimations of the development of the phagocytic substances furnish the best indications as to the value of a typhoid vaccine, a conclusion they have reached as a result of their numerous experiments, many of which have been described above, and for which they find some confirmation in the results of this particular experiment.

Owing to the small number of cases classed as mild there was little information to

[1] Journal of the Royal Army Medical Corps, Vol. XI., page 327, 1908.

be gained as to the relationship of the different protective substances to the severity of the original attack. On the whole, it would appear that in the prolonged and severe cases there is a diminished production of these substances and that this deficit is not compensated by increased production at a later stage of convalescence. This appears in accordance with the personal observations of several of the investigators that men who have recovered from an exceptionally severe attack of enteric fever have little protection against another attack and, indeed, appear hyper-sensitive and likely to contract a fresh infection.

Finally, although the fatal case was an isolated one, the blood examination brought out the fact that, although the agglutinins were present in moderate amount and the bactericidal power was good, at the time the sample was tested, the opsonins were definitely in default and even lower than the amount found in normal individuals. This, if confirmed in similar cases, would furnish a point of considerable prognostic significance and, in addition, would bear further witness to the essential nature of the part played by phagocytosis in combating an attack of enteric fever.

PART II.

CHAPTER I.

The Statistical Results of Inoculation.

One of the earliest proposals of the Committee was that a medical officer should be attached to each regiment proceeding to a foreign station for a period of 3 years, with a view to the collection of accurate statistical information as to the results of inoculation.

Their recommendations on this subject were agreed to by the authorities, and, from the end of 1904 to the beginning of 1909, a medical officer was attached for this purpose to each of the regiments of Cavalry or Infantry or brigades of Artillery which have left this country for foreign or colonial service. In this part of the report, the information obtained in connection with these units is set forth.

The reasons for the proposal were based upon the difficulties which the Committee found in interpreting with accuracy the tables of statistics presented to them at their first meetings which had been prepared from official or private sources without any definite and general plan. Many of these earlier tables, and also the later official returns, although full of interest, did not give the detailed information which they felt to be essential. Much of the information which they had in view was of such a nature that it was obvious it could only be obtained by means of careful records kept by an officer who should have the unit under his personal observation for a period of years. The nature of the observations which were to be made by such attached officers is detailed below.

The scheme has been carried out to the satisfaction of the Committee, and the Committee feel that the information which they have obtained through it is of great value and throws light upon many of the points upon which they desired particulars.

The system adopted has been as follows. Some months before the commencement of each trooping season, *i.e.*, in June or July, a medical officer has been detailed by Army Headquarters for attachment to each of the units under orders to proceed on foreign service during the ensuing season. These officers were Lieutenants of 2 to 3 years' service and they were selected for their special duties on the recommendation of Colonel Leishman, through whose class they had recently passed. Each of these officers was then instructed to attend at the Laboratories of the Royal Army Medical College for a period of 10 days, during which he received further training in the bacteriological work which would subsequently be called for in connection with his special duties. During this 10 days' laboratory work, the officers were thoroughly instructed in modern methods of isolating and identifying the typhoid bacillus and the allied members of this group of organisms, in the technique of agglutination work, and in the preparation and employment of typhoid vaccine.

The following is an outline of the instructions given to them in connection with their special duties:

1. They are to be attached to the unit for a period of 3 years and are to accompany it on all its changes of station during this period.
2. They are to carry out the inoculations in their unit, both before embarkation and after their arrival at their station abroad.
3. They are to keep accurate records of all men inoculated, with full particulars of the vaccine used, date, dose, &c., and full detailed information of all cases of enteric fever, whether occurring among the inoculated or the non-inoculated.
4. They are to keep in touch with all men detached from the headquarters of the unit, in order to make sure that no case of real or suspected enteric escapes observation and record.
5. If not placed by the Senior Medical Officer of the station in actual charge of the fever cases, they are to arrange that they obtain full particulars of such cases, and that they have access to them for the purpose of carrying out the blood tests in the manner in which they have been instructed.

6. They are to verify the diagnosis of enteric fever by bacteriological methods wherever possible.
7. They are to perform, or at least to attend, all post-mortem examinations on men dying of enteric fever, to record the post-mortem appearances in such cases and to make cultures in the way they have been shown.
8. They are to arrange for the effective carrying out of their duties if sick or on leave.
9. For the information of the Anti-typhoid Committee, they are to keep Lieut.-Colonel Leishman posted in all that bears upon their special duties and to send him from time to time such returns as may be called for by him, showing the influence of inoculation upon the incidence, severity, mortality, &c., of enteric fever in their units.

The duties indicated in the above instructions have been well carried out by the officers concerned, and the results of their reports have been analysed by Colonel Leishman and appended as a short history of the events concerned with inoculation in each of the 24 units. A table summarizing the results of the whole of the units is also appended.

Before commencing the account of each individual unit it may be well to point out that in certain instances the whole of the information required could not be obtained owing to the exigencies of military service and, at times, such information could only be obtained by the medical officers under conditions calling for the greatest tact and judgment. That the reports are as complete and detailed as will be seen to be the case reflects, in Sir William Leishman's opinion, the greatest credit on the officers concerned, and with this the Committee, after a careful study of the returns, is in full agreement.

Many of the difficulties alluded to would only be fully appreciated by those familiar with the life and surroundings of the soldier and will not be mentioned again, except where necessary to explain some blank in the information furnished by the attached medical officers.

Unit No. 1.

2nd Bn. The Royal Fusiliers.

Medical Officer—Captain A. B. Smallman, R.A.M.C.

This regiment, as will be seen from the section of the report dealing with the experimental work,[1] was the one which was selected in the autumn of 1904 as the first of the "Test-units," and it was in connection with it that the daily analysis of the protective substances appearing in the blood after inoculation was carried out. Captain Smallman was selected to proceed abroad with them for 3 years as he had been one of the officers who carried out this experimental work at Aldershot, and the Committee desired that further tests of the same nature should be carried out after the regiment had arrived in India.

For this purpose Captain Smallman was provided with a bacteriological outfit suitable for the carrying on of the tests in which he had already been engaged, and on reaching the first Indian station of the regiment he proceeded, as early as practicable, to recommence this analytical work. The results of this work, both that which preceded and that which followed the departure of the regiment from England, have already been considered in Chapter III, and need not be further mentioned here, except with reference to the cases of enteric fever which subsequently occurred.

History and movements of the unit.—Captain Smallman joined the battalion at Aldershot on 16th October, 1904, and accompanied it to India. The regiment sailed from Southampton on 23rd November and arrived at Calcutta on 23rd December. They encamped on the Maidan, outside Fort William, and remained there until 3rd January, 1905, when they proceeded by rail and route march to Lebong, which was reached on 9th January. This station adjoins Darjeeling and has an altitude of about 7,000 feet. It was unfortunate, from the point of view of the experiment, that it is one of the healthiest stations in the hills, and that enteric fever is practically unknown there.

The regiment left Lebong on the 5th November, 1905, and proceeded to their first station in the plains, Trimulgherry, adjoining Secunderabad, which was reached on 16th November, 1905. The barracks being full they remained under canvas at the station until the middle of February, 1906, when they occupied the barracks vacated by the regiment which they had relieved.

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[1] See Chapter III., page 7.

Captain Smallman's connection with the regiment ceased at the end of December, 1907, on his completing the 3 years originally fixed, and he was transferred to Quetta to take up the appointment of Divisional Sanitary Officer at that station.

Inoculations.—The strength of the battalion at the time of embarkation for India was very low, 358 (all strengths here mentioned refer to warrant officers, non-commissioned officers and men only and do *not* include officers, women or children), the battalion being made up to full strength by drafts from its other battalion after it had left England. 106 men presented themselves for inoculation at Aldershot and of these 86 received two doses. This number may seem small, but it must be recalled that this was the first regiment to be addressed on the subject of inoculation after it had been in abeyance in the Army for nearly 2 years, and further that, owing to furloughs and other causes, no more than 200 men could be collected to listen to Colonel Leishman's appeal.

The men who subsequently joined the battalion included some who had been inoculated during the period of the South African War, 5 –6 years before, and a certain number of fresh inoculations were carried out by Captain Smallman while he was with the regiment, as well as some re-inoculations of men previously inoculated at Aldershot. He was not, however, very successful in this and during his 3 years he only succeeded in raising the inoculated strength from 106 to 198.

Owing to the fluctuations constantly going on in the personnel of a regiment it will be manifest that the relative proportions of inoculated to non-inoculated must oscillate from month to month. Men transferred to other corps, discharged as time-expired, deaths from any cause, &c., reduce the numbers of one or other group, while fresh arrivals increase them. Such fluctuations are inevitable, and it was thought that the clearest idea of the actual state of affairs in a given unit would be to present the figures under three separate heads which record, first, the inoculated strength on arriving at the foreign station, second, the total number of fresh inoculations carried out by the medical officer during his period of observation, together with other additions to the strength of the inoculated group, and, third, the actual strength of the inoculated present with the unit on the date of the last report received from it. A similar principle has been adopted with regard to the total strength of the unit, and the following figures are given in each instance: first, the strength (always excluding officers, women and children) at the date on which the medical officer joined the unit; second, the strength when the unit was brought up to or near its full establishment on reaching its foreign station, and, third, the strength of the unit on the date at which the last report concerning it was dispatched. It may be explained that the second of the columns, the one giving the strength on completing the establishment at the foreign station, is necessary on account of the fact that many battalions take over a large number of men from their "linked" battalion at such a time, if the latter happens to have been serving in the same country and to be on the point of returning to England.

The alternative method of presenting the figures in the form of averages appeared less likely to be readily interpreted and would have entailed great additional labour on the returning officers without, apparently, any great advantage either in accuracy or simplicity.

Incidence of enteric.—As already noted, it was unfortunate, from the point of view of judging the protective value of the inoculations, that the regiment should have been posted in so healthy a station. The only cases which occurred while they were at Lebong were two uninoculated men, who were admitted to hospital on 21st and 22nd September, 1905, respectively. In their case the disease was not contracted in the station, but either in Allahabad or on the way from that place to Lebong; they had been detached to that station to undergo a Mounted Infantry course. Both cases were mild, and recovered. Another case was admitted soon after, but Captain Smallman was able to determine that it was a para-typhoid infection; the latter also was an uninoculated man. It should be mentioned that, where clear evidence was forthcoming, cases of para-typhoid fever are not included in the returns, either of the inoculated or of the non-inoculated.[a]

On changing their station to Trimulgherry, however, enteric fever was not long in making its appearance in the regiment, the first case being admitted on the 1st February, 1906, and 42 cases in all being diagnosed during that year. Of these nine occurred in inoculated men and 33 in non-inoculated. Eight of the 42 terminated fatally, one of them being an inoculated man. Three of the eight deaths, however, are

[a] See remarks in connection with Unit No. 2, page 61.

not shown in the returns as deaths from enteric as they died from cholera. This disease broke out in August in the enteric fever ward; 13 men, including three of the Fusiliers, were attacked, all of whom died except one. Two of the cholera deaths were in men who had not been inoculated, the third in an inoculated man.

Captain Smallman, commenting on the incidence of enteric during this year, noted that there appeared to be little or no evidence of protection in the case of the inoculated men, the case-incidence of the two groups being almost identical. In connection with this, however, it must be recalled that the first case in an inoculated man did not take place until more than 15 months after inoculation, and that it had been subsequently proved by the experimental work detailed in Part I., that the particular vaccine used in this regiment was one which had lost a large part of its protective power owing to the manner of its preparation. The vaccine prepared in this manner will be alluded to throughout as the "old vaccine" to distinguish it from the modified vaccine since employed. Captain Smallman's analytical work at Lebong bears further witness to the accuracy of the conclusion founded on the experiments conducted at the Army Medical College, as he found that in 4—6 months the protective substances had fallen to the normal values in the blood of the inoculated men. Unfortunately, the results of the work alluded to were not arrived at until a later period, so that the fresh inoculations and re-inoculations which were carried out by Captain Smallman were done with the same "old vaccine," which must now be looked upon as capable of giving but transitory protection in comparison with that conferred by the vaccine with which subsequent units were inoculated.

During 1907 the regiment remained at Trimulgherry and in this year 25 cases occurred among the non-inoculated, with four deaths, and there were also two cases among the inoculated, one of whom died. All of the men who had been inoculated had been done with the old vaccine.

During this year Captain Smallman carried out 23 fresh inoculations and re-inoculations, and, although the number is small, it was noted that no case of enteric occurred among these men, the first in the regiment to receive the new vaccine.

At the end of this year (1907) the 3 years of observation were concluded, and Captain Smallman was posted to another station. On the 31st December, 1907, the result as regards inoculation and enteric during this period stood thus—

Total strength of regiment	1,013
Number of inoculated	198
Number of non-inoculated	815
Cases of enteric in inoculated men	11
Deaths from enteric in inoculated men	1
Cases in non-inoculated men	60
Deaths in non-inoculated men	9

Since Captain Smallman left the regiment, however, later information was received as to the incidence of enteric fever during the following 6 months, the figures during this period have therefore been included in the general return, and they bring the total number of cases up to the following points:—

Résumé.

Total strength of regiment	1,013
Number of inoculated	198
Number of uninoculated	815
Total number of cases	80
Total number of deaths	10
Cases in inoculated men	12
Deaths in inoculated men	1
Cases in non-inoculated men	68
Deaths in non-inoculated men	9

Case incidence.

Inoculated	60 per 1,000
Non-inoculated	83 „

Case mortality.

Inoculated	8·3 per cent.
Non-inoculated	13·2 „

The whole incidence, therefore, in the case of the Royal Fusiliers refers to incidence in men inoculated with the "old" vaccine.

In his report on the nature of the cases Captain Smallman states that he was quite convinced that those which occurred among inoculated men were of a milder nature and of shorter duration than those which occurred among the non-inoculated, and this is borne out by the case sheets and temperature charts submitted for examination.

Unit No. 2.

The 17th Lancers.

Medical Officer.—Captain E. J. Luxmoore, R.A.M.C.

The medical officer attached to this and the subsequent units received special instruction at the Royal Army Medical College in his special duties in connection with inoculation and its results, but he was not trained in the analytical methods of measuring the protective substances, other than the agglutinins, and was not provided with the laboratory apparatus with which Captain Smallman was furnished. The tests which Captain Luxmoore and his successors carried out were conducted at the nearest station or district laboratory, either by themselves or, at times, by the officer in charge of the laboratory.

History and movements of the unit.—The 17th Lancers were stationed at Edinburgh prior to their embarkation for India and before Captain Luxmoore joined them in August, 1905, Colonel Leishman visited the headquarters of the regiment in order to discuss the question of inoculation with the Commanding Officer. It must be borne in mind that, in the case of these first units, there was considerable difficulty in getting the men to come forward voluntarily as inoculation had been so long in abeyance and little response could be looked for in the absence of support from the Colonel and the officers of the corps concerned. In this instance the regimental authorities gave their cordial co-operation, and Captain Luxmoore after joining the regiment was able to inoculate 130 men out of a strength of 490 prior to embarkation.

The vaccine used in this instance was the "new" vaccine, as the results of the experiments at the Royal Army Medical College had by this time shown that the earlier method of preparation diminished to a considerable degree the immunising properties.

The Lancers sailed in September, 1905, and reached their station, Meerut, on 4th October, 1905. They remained at this station during the whole of the 3 years during which they were under observation.

As Captain Luxmoore remained at the station after the expiry of his 3 years' appointment, and was still in touch with the regiment, the report brings its history up to July, 1909, a period of observation of 3 years 10 months.

Inoculations.—As mentioned above, 130 men were inoculated out of a strength of 490 before sailing. Very soon after reaching Meerut, indeed in the month following, a severe epidemic of enteric attacked the regiment, and the incidence of the disease being almost exclusively confined to those who had not been inoculated naturally proved of great help in the subsequent endeavours of Captain Luxmoore to increase the number of the inoculated. During the period of 3 years during which he was attached to the Lancers he carried out no less than 433 fresh inoculations and 263 re-inoculations. At the date of the last return from the regiment, dated 9th August, 1909, there were present 460 inoculated men out of a strength of 620.

In addition to the number of men protected by inoculation there are always present a certain number of soldiers who have suffered from enteric fever in the past. In the case of this regiment, in particular, this group was a gradually increasing one and eventually attained considerable size owing to the very large number of cases which occurred during the period of observation. This is a factor which to some extent complicates all returns of inoculation concerning soldiers and it was partly for this reason that a limit of 3 years was fixed upon as the maximum period during which it was unlikely to interfere with the general statistical observations to any great extent. Whatever

influence this has on the figures it was decided not to attempt a statistical record of it in the case of these units as it was particularly desired to keep them as simple and easy of interpretation as possible. Finally, it may be neglected the more safely as any influence which it might be shown to have on the figures would be to the disadvantage and not to the advantage of inoculation, since men who have had enteric in the past and who have never been inoculated are, in a sense, protected men, who go to swell the numbers of the uninoculated.

However, in this case of the 17th Lancers, in which the number of cases of enteric has been much the largest of any of the 24th units, and in which, in consequence, the number of the men shown as non-inoculated in the returns comprises a larger proportion of men protected by a previous attack than in any other unit, Captain Luxmoore worked out the following tables. In these it will be seen that he deals with averages and not actuals as regards the strengths. These averages were obtained by adding together the number of days in the year for which each man was, as the case may be, protected by one, two or more doses of vaccine, unprotected by inoculation or protected by a previous attack of enteric fever; the result was then divided by the number of days in the year.

TABLE I.

Averages for the first 12 months in India.

Average annual strength 562.	Strength.	Admissions for enteric.	Deaths from enteric.
Average number of men unprotected by inoculation or previous attack of enteric.	373	64	11
Average number of men protected by one dose only	33	2	0
Average number of men protected by two or more doses.	110	0	0
Average number of men protected by previous attack of enteric.	46	0	0

TABLE II.

Averages for the second 12 months in India.

Average annual strength 616.	Strength.	Admissions for enteric.	Deaths from enteric.
Average number of men unprotected by inoculation or previous attack of enteric.	238	6	1
Average number of men protected by one dose only	35	0	0
Average number of men protected by two or more doses.	287	0	0
Average number of men protected by previous attack of enteric.	56	0	0

Incidence of enteric.—The disease appeared in the regiment within a few weeks of their arrival at Meerut, and 66 cases with 11 deaths had occurred in the period of one year from their arrival at Meerut.[1] It will thus be seen that the regiment presented a marked contrast to that which has just been described, the Royal Fusiliers, inasmuch as it was attacked by enteric within a few weeks of the carrying out of the first inoculations and a greater part of the incidence of the disease occurred within a year after inoculation. Another point of difference lies in the fact that in this regiment the "new" vaccine was employed for the first time, as the experiments already referred to had been concluded shortly before the departure of the Lancers from England.

The occurrence of the epidemic therefore afforded the opportunity of determining, to some extent, the value of the modifications introduced in the preparation of the vaccine and its protective properties in the case of men who had, presumably, reached the highest point of their immunization.

[1] A detailed account of the greater portion of this epidemic, 60 cases, has been published by Captain Luxmoore in the Journal of the Royal Army Medical Corps, Vol. VIII., page 492, 1907.

The early effects of inoculation in the regiment may be readily appreciated by the study of the following table, which records the results reported by Captain Luxmoore from the arrival of the regiment in India up to the 1st May, 1907, a period of 1 year and 7 months. Under each date is given the total number of cases and deaths which had occurred in each group up to that date:—

17th Lancers—Arrived Meerut, 4th October, 1905.

Date.	Strength of regiment.*	Inoculated.			Non-inoculated.		
		Strength.	Cases.	Died.	Strength.	Cases.	Died.
4th December, 1905 ..	592	172	1	0	420	36	5
5th January, 1906 ..	614	174	1	0	440	46	6
30th September, 1906 ..	680	258	2	0	422	66	11
30th December, 1906 ..	676	396	2	0	280	68	11
1st May, 1907	628	425	2	0	203	69	11

The above table covers the period of the greatest incidence of enteric in the regiment and there were only a few more cases during the remainder of the year 1907. In the following year, 1908, cases occurred at intervals throughout the year and on the 30th December, *i.e.*, 2 years and 3 months since their arrival in India, the figures stood as follows. (Officers are not included and the strengths are the actual strengths then present with the regiment and are not average strengths):—

Strength of regiment	650
Number of inoculated	430
Number of uninoculated	220
Cases of enteric in inoculated men	13
Deaths from enteric in inoculated men	1
Cases of enteric in uninoculated men..	95
Deaths from enteric in uninoculated men	13

Although Captain Luxmoore's period of attachment to the regiment terminated on 4th October of this year he remained on duty at Meerut and was therefore able to furnish later information as to the further incidence of the disease and in the last return furnished by him on 9th August, 1909, the results stand as follows:—

Résumé.

Strength of regiment	620
Number of inoculated	460
Number of uninoculated	160
Cases of enteric in inoculated men	18
Deaths from enteric in inoculated men	2
Cases of enteric in uninoculated men..	96
Deaths from enteric in uninoculated men	18

Case incidence.

Inoculated	39·1 per 1,000
Non-inoculated	600 „

Case mortality.

Inoculated	11·1 per cent.
Non-inoculated	18·7 „

In this regiment, therefore, not only has the incidence of enteric fever been exceptionally severe, but the ratio of the cases occurring in the inoculated to those in the non-inoculated stands, at the end of the 3 years and 10 months' observation, at the remarkable figure of 1 : 15.

From the case mortality in the non-inoculated it is evident that the type of the disease was of the average severity encountered in India and, apart from this, the diagnosis has been verified as far as possible by agglutination work and, during recent

* In the above returns officers are included in the strengths, in all other returns they are excluded.

years, by blood cultures during the attack. As a result of the systematic attempts made to isolate the causative bacillus a certain proportion of cases of para-typhoid infection were encountered, but since these examinations were not found to be practicable during the first year of observation and both groups may therefore have included a certain number of such para-typhoids, the cases of this disease subsequently encountered have been allowed to remain in whichever group they occurred. In this connection, however, it must be borne in mind that the experimental work detailed in the earlier section demonstrated that typhoid vaccine had little or no protective effect against para-typhoid infections; the inclusion therefore of certain cases of this infection in the total of the cases occurring in inoculated men is unfair to inoculation. In the subsequent units such para-typhoid infections, as has already been mentioned, have been excluded from both groups when the evidence was complete.

A few more points of interest emerge from a consideration of the details of the cases which occurred in inoculated men.

The smaller degree of immunity conferred by a single dose of vaccine as contrasted with that resulting from two doses appears manifest, since no less than six of the whole 18 cases which occurred in the inoculated were in men who had refused to have their second dose, and who, in consequence, had received only one-third of the amount of vaccine given to the others. This was especially marked during the first two years during which the only cases which occurred in inoculated men, two in number out of a total of 72 during this period, were in men who had only had one dose.

The age and nature of the vaccine used in the cases which contracted enteric.—Two of the cases in the inoculated had been done with a vaccine prepared at the Pasteur Institute, Kasauli, and, although they remain in the return, these should not strictly have been included in a table designed to elucidate the effects of the vaccine prepared at the Royal Army Medical College. In three other cases the vaccine used was of greater age than has since been shown to be effective, and in one of the fatal cases the attack took place 3 years and 1 month after inoculation; it is probable that little or no protection can have been left after this time.

To summarise the analysis of the 18 cases in the inoculated men there have, during the 3 years and 10 months, been only eight cases in men who received two or more doses of vaccine prepared at the Royal Army Medical College which had not been inactivated by age. Two of these cases were exceedingly mild, as the fever only lasted eight days and ten days respectively. Two others were reported as being "mild," one was a case of moderate severity and another was an undoubted case of paratyphoid, as this bacillus was isolated from the blood during life. The two remaining cases were fatal, but one of these men was attacked while he was still under treatment for a long and debilitating gonorrhœa, and in the case of the other man a period of 3 years, 1 month had elapsed between his inoculation and attack.

Unit No. 3.

12th Brigade, Royal Horse Artillery.

Medical Officer—Captain E. G. R. Lithgow, R.A.M.C.

History and movements of the unit.—This brigade was constituted by batteries "V" and "W" of the Royal Horse Artillery at Aldershot, on the 16th of December, 1905. Captain Lithgow joined them at this station and accompanied them to South Africa, where they were quartered at Pretoria, which was reached by the brigade on 20th January, 1906. The unit remained there as a brigade until the end of October, 1907, when they proceeded from South Africa to India, arriving at Bombay on the 9th of November, 1907. The brigade was then broken up, "V" Battery proceeding to Rawal Pindi, and "W" Battery to Sialkot. Captain Lithgow accompanied "V" Battery to Pindi, and kept touch, as far as possible, with "W" Battery in order to keep up the records. It will be realized, however, that the difficulties in connection with the records were greatly increased by this dissociation of the two batteries.

Under instructions from the War Office, Captain Lithgow's connection with the unit was terminated on 1st July, 1908, and the returns summarised below are therefore not carried beyond this date, *i.e.*, they cover the inoculation history of the unit for a period of 2 years and 7 months.

Inoculations.—Captain Lithgow's endeavours to persuade the men of these batteries to be inoculated before leaving Aldershot met with but a poor response, and only 40 men were done before the brigade sailed. During the year and 9 months during which

the brigade remained in South Africa, also, he was only able to carry out 20 fresh inoculations. A fresh impetus was given, however, on the brigade reaching India, 131 fresh inoculations being carried out in "V" Battery at Rawal Pindi, and 31 in "W" Battery at Sialkot. The last return on 30th June, 1908, showed, out of a strength of 330 in the two batteries, that there were 202 inoculated men.

Incidence of enteric.—This has been slight in the brigade, only seven cases with one death having occurred during the 2½ years they were under observation. The whole of the cases occurred in the uninoculated men.

The disproportionate size of the groups of inoculated and non-inoculated while the brigade was in South Africa, 60 men only being inoculated out of a strength of about 350, discounts to a certain extent the apparently excellent results of inoculation, but, in any case, the number of cases in this period is too small to be of much value.

The final results, then, stand as follows:—

Résumé.

Strength of brigade	330
Number of inoculated	202
Number of uninoculated	128
Cases of enteric in inoculated men	0
Deaths from enteric in inoculated men	0
Cases of enteric in uninoculated men	7
Deaths from enteric in uninoculated men	1

Case incidence.

Inoculated	Nil.
Non-inoculated	54·6 per 1,000

Case mortality.

Inoculated	Nil.
Non-inoculated	14·2 per cent.

Unit No. 4.

14th (King's) Hussars.

Medical Officer—Captain C. E. Fawcett, R.A.M.C.

History and movements of the unit.—The 14th Hussars were stationed at Shorncliffe when Captain Fawcett joined them on the 1st August, 1906. They sailed for India at the beginning of the following month and arrived at Bangalore on the 30th of September. They had no change of station during the period which they were under observation, and at the date of the last report, 8th July, 1909, they were still quartered in Bangalore.

Inoculations.—Out of a total strength of 558 the regiment arrived at Bangalore with 244 inoculated men. Fresh inoculations were carried out as opportunity offered in India, and on 8th March, 1907, Captain Fawcett reported that the number had been raised to 310. At the end of 1908 there were 421 inoculated men present with the regiment out of a strength then present of 546, and Captain Fawcett had, in all, carried out 187 fresh inoculations, but no reinoculations. The balance of the inoculated, unaccounted for by Captain Fawcett's original and subsequent inoculations was made up by men transferred to the regiment who had been inoculated in other stations.

On the 8th July, 1909, the date of the last return, there were present with the regiment 544 men, of whom 377 had been inoculated. The fall in the number of the inoculated is accounted for by the departure from the regiment of time-expired men, and by the fact that Captain Fawcett had not carried out any fresh inoculations during the first 6 months of the year 1909.

Incidence of enteric.—The 14th Hussars suffered little from enteric, there having been only 10 cases during the 2 years and 10 months. The first case occurred in February, 1907, and this was the only one admitted to hospital during the first year of the regiment's stay in India. The case was in a non-inoculated man and it terminated fatally.

During 1908, eight cases occurred; four in men who had been inoculated and four in non-inoculated, one of the inoculated cases died from perforation on the 27th day, when he appeared to be progressing favourably. Captain Fawcett reported that the other three cases in the inoculated were very mild. Diagnosis was made on the clinical signs and on agglutination. None of the cases reacted to paratyphoid.

During 1909 there was only one other case, in a non-inoculated man, and this case also proved fatal.

The only comment which appears called for in connection with the four cases occurring in inoculated men is the length of time elapsing since the inoculation. Each of the four had received two doses of the "new" vaccine, but the dates of their attacks show that the following periods had elapsed in their respective cases between inoculation and attack: 336 days, 375 days, 498 days and 666 days. The man who died had been inoculated nearly 2 years before.

Résumé.

Strength of the regiment	631
Number of inoculated	377
Number of uninoculated	257
Cases of enteric in inoculated men	4
Deaths from enteric in inoculated men	1
Cases of enteric in uninoculated men	6
Deaths from enteric in uninoculated men	2

Case incidence.

Inoculated	10·6 per 1,000
Uninoculated	23·3 „

Case mortality.

Inoculated	25 per cent.
Uninoculated..	33·3 „

Unit No. 5.

3rd Bn. Coldstream Guards.

Medical Officers—Lieutenant J. H. Graham, R.A.M.C.; (later) Major W. D. Erskine, R.A.M.C.

History and movements of the unit.—This battalion of the Guards was joined by Lieutenant Graham at London, on 29th September, 1906, and they sailed shortly afterwards for Egypt, arriving at Abbassieh, near Cairo, on 11th October, 1906. The regiment remained there until the end of 1908, when the headquarters proceeded to Khartoum, which was reached on 1st November, 1908, two companies being detached to Alexandria on 20th October, 1908. The regiment remained at these latter stations at the date of the last report, received from Major Erskine on 23rd August, 1909.

Lieutenant Graham, at the beginning of 1908, was directed to do duty at the Citadel, Cairo, and the medical charge of the Guards fell to Major Erskine who was at Abassieh; later in the year the former officer was ordered to Gibraltar, and from that time Major Erskine continued to carry out the work in connection with inoculation, and he accompanied the battalion to Khartoum.

Inoculations.—Before sailing, about 90 of the men were inoculated, but time did not permit of all having their second doses before sailing, so it was arranged that they should have this done on the voyage out. The voyage was, however, so rough that the men refused *en masse* to have this done, and on arrival at Abassieh only 46 men had their inoculations completed. The remainder, however, received their second inoculations at Abassieh about one month after their first doses, and Lieutenant Graham wrote on 3rd November, 1906, that he had got 90 fresh volunteers, raising the total number of men inoculated to 180. From time to time further inoculations were done, and at the end of their first year the number had been raised to 293.

During their second year, Major Erskine was very successful in persuading the men to come forward, and out of a strength of 706 on 31st July, 1908, there were 514 inoculated men to 192 non-inoculated.

At the end of December, 1908, out of a strength of 754, there were 540 inoculated men and these numbers remained unchanged in the last report, dated from Khartoum on the 23rd August, 1909.

Incidence of enteric.—At the beginning of 1907 enteric began to appear in the regiment when quartered at Abassieh, four cases, all in uninoculated men, being reported up to the end of April. On 10th November of this year the number had increased to a total of 12 cases, one of which proved fatal, and all of them, with the exception of one very mild case in an inoculated man, were men who had refused inoculation. Two of the cases, however, which were reported as occurring in the non-inoculated, were married women of the regiment and are therefore not included in the Return. From November, 1907, the cases ceased and the regiment remained clear of enteric for the next 7 months. In the autumn of 1908 four more cases occurred, one in an inoculated man, and the other three in uninoculated men. Both cases were mild, that in the inoculated man exceptionally so, the pyrexia only lasting 12 days. From this time to the date of the last return no fresh cases had occurred.

Major Erskine, as mentioned, took great trouble to increase the number of the inoculated at Abassieh, not only in the Coldstream Guards but in the other units quartered there, namely, the 6th Dragoons and "A" Battery, Royal Horse Artillery, and he was greatly impressed with the protective effects of inoculation. Writing on the 7th July, 1908, he says, "Only two cases of enteric fever have been reported in this large garrison for the 6 months ending 30th June, both uninoculated." This station, Abassieh, was formerly one of the chief foci of infection in Egypt, and Major Erskine, as the result of his experience there, said that he was inclined to look upon typhoid inoculation in much the same light as he looked on vaccination against small-pox.

No further cases occurred among the Guards at Khartoum and the figures on the 23rd August, 1909 stand thus:—

Résumé.

Strength of the regiment	754
Number of inoculated	540
Number of uninoculated	214
Cases of enteric fever in inoculated men	2
Deaths from enteric fever in inoculated men	0
Cases of enteric fever in uninoculated men	10
Deaths from enteric fever in uninoculated men	1

Case incidence.

Inoculated	3·7 per 1,000
Uninoculated	46·7 "

Case mortality.

Inoculated	Nil.
Uninoculated	10 per cent.

Unit No. 6.

2nd Bn. Leicestershire Regiment.

Medical Officer—Captain H. G. Sherren, R.A.M.C.

History and movements of the unit.—Captain Sherren joined this regiment at Colchester on the 6th August, 1906. It sailed for India in the middle of September of that year and reached Belgaum on the 13th October. There were no changes of station during the period under observation, and the latest report, dated from Belgaum on 8th July, 1909, covers a period, therefore, of 2 years 9 months in the same station.

Inoculations.—The battalion when it sailed from England mustered only 356 men as it was to make up its full strength from its linked battalion on its arrival in India. At Colchester, therefore, and on the voyage out, Captain Sherren was not able to carry out very many inoculations and on reaching Belgaum the inoculated strength was only 149. Of the men of the other battalion who joined the 2nd Battalion in India he found out of a total of nearly 500 that only 39 had been inoculated. His earlier efforts to increase the numbers were not very successful, doubtless on account of the presence of so many old soldiers in the regiment. Later he met with more success, especially when enteric began to appear and, by the middle of the following year, 1907, out of a strength of 963 there were 346 inoculated.

In the next year, 1908, the men showed considerably less opposition and, by the end of that year, the numbers of the inoculated had been raised to 575 out of a strength then present of 935. At the date of the last report, 8th July, 1909, out of 910 men 589 had been inoculated.

Altogether, during the period under observation Captain Sherren has carried out 568 fresh inoculations and 110 re-inoculations, the discrepancy between the total and the number present with the battalion at the last report being accounted for by the inoculated men who had left the regiment, time-expired or as transfers to other units, and by deaths from other diseases.

Incidence of enteric.—No cases occurred in the regiment for the first 9 months of their being stationed in India, but since then the incidence, although not heavy, has been fairly steady. Between June, 1907, when it first appeared, and 14th March, 1908, there were 20 cases with two deaths. Three of these 20 cases were in inoculated men and one of these died. At the end of December of that year, 1908, out of a strength of 935, of whom 575 had been inoculated, there had, in all, been 26 cases, five of these having been in inoculated men, with one death, while two of the 21 non-inoculated men died.

The last report, on 8th July, 1909, showed a total of 30 cases, 24 in the non-inoculated with three deaths and six in the inoculated with one death. The strength of the regiment at this time was 910 men, of whom 589 had been inoculated.

Of the six cases in the inoculated it is to be noted that four had been done with the "old" vaccine, *i.e.*, a vaccine similar to that used in the case of Unit No. 1, the Royal Fusiliers and, presumably, had only received a short-lived immunity from this. The fatal inoculated case was not one of these, but in his case there had been an interval of more than 4 months between his first and second inoculation instead of the 10 days which is considered the appropriate time.

As regards the five inoculated cases which recovered, two are classed as "very mild," two as "mild" and the fifth is not included in the returns as the man was inoculated in the incubation stage of the disease; his case is, however, of interest in view of the supposed dangers of the negative phase in such an event; instead of running a severe course the attack was exceptionally mild and the duration of the pyrexia was only 9 days. It will be seen, however, that a similar case in connection with Unit 21 terminated fatally.

Résumé.

Strength of the regiment	910
Number of inoculated	589
Number of uninoculated	321
Cases of enteric in inoculated	5
Deaths from enteric in inoculated	1
Cases of enteric in uninoculated	24
Deaths from enteric in uninoculated	3

Case incidence.

Inoculated	8·4 per 1,000
Uninoculated	74·7 „

Case mortality.

Inoculated	20· per cent.
Uninoculated	12·5 „

Unit No. 7.

2nd Bn. Dorsetshire Regiment.

Medical Officer—Captain E. G. Anthonisz, R.A.M.C.

History and movements of the regiment.—This battalion of the Dorsetshire Regiment was stationed at Colchester when Captain Anthonisz joined them on 14th August, 1906. They left England at the beginning of October of that year, and arrived at Wellington in Madras on 2nd November, 1906. The regiment remained at Wellington for the next 2 years, the headquarters being fixed in this station while detachments were furnished to the three following stations: Cannanore, Calicut and Mallapuram

At the end of these 2 years the battalion moved to the City of Madras, reaching this on the 2nd November, 1908, exactly 2 years after they had arrived at Wellington. Here, also, the regiment was divided, four companies being detached to be stationed at Bellary. On the date of Captain Anthonisz's last return, 6th June, 1909, this was still the distribution of the battalion.

Inoculations.—The battalion on leaving England numbered only 376 men, as it was to take over a draft of 600 men from the 1st Battalion, at that time on the point of leaving Ferozepore, on their arrival at Bombay. Captain Anthonisz found it very difficult to persuade the men to volunteer for inoculation, and, on their arrival in India, the total strength of inoculated (excluding, as usual, the officers) was only 62. Only a few of the 600 men who had been taken over from the 1st Battalion had been inoculated. On arrival at Wellington his further efforts also met with but little success; Wellington being a very healthy station, 6,000 feet above the sea level, and almost always free from enteric, it was not difficult for the men to persuade themselves that they ran no danger, and, in the absence of any strong support from the officers of the regiment, Captain Anthonisz's early difficulties were comprehensible. By degrees, however, the numbers were increased, especially when fresh drafts of younger soldiers joined the regiment from England during the following trooping season, and, at the beginning of 1908, the number of the inoculated had been raised to 196 out of a total strength of 1,107; 22 of these men, however, had only received one inoculation, refusing to come forward for their second dose.

At the end of 1908 the number of the inoculated was 255 out of a strength then present of 1,020.

When stationed at Madras Captain Anthonisz was able to get a few more volunteers, and, at the date of his last report on 30th June, 1909, the numbers were 311 inoculated out of a strength of 1,108.

Incidence of enteric.—There was very little enteric during the first 18 months of the regiment's stay in India. At the end of April, 1908, there had been, in all, six cases, four of which occurred at Wellington and two at Calicut. None of these were fatal. Some more cases occurred, however, on the regiment moving to Madras and Bellary, and on the 17th December, 1908, there had been a total of 12 cases with one death. In his last report of 30th June a further fatal case was recorded, bringing the total number of cases occurring during the 2 years and 7 months to 13 with two deaths.

With one exception the whole of the 13 cases were in men who had refused inoculation. The single case in an inoculated man was one of those who had refused to come forward for his second inoculation. The case was reported as "very mild," and the man recovered without relapse or complication. The following is the final return:—

Résumé.

Strength of the regiment	1,108
Number of inoculated	311
Number of uninoculated	797
Cases of enteric in inoculated	1
Deaths from enteric in inoculated	0
Cases of enteric in uninoculated	12
Deaths from enteric in uninoculated	2

Case incidence.

Inoculated	3·2 per 1,000
Uninoculated	15· „

Case mortality.

Inoculated	0·0 per cent.
Uninoculated	16·6 „

UNIT No. 8.

1st Bn. Connaught Rangers.

Medical Officer—Captain A. O'Carroll, R.A.M.C.

History and movements of the regiment.—The regiment was stationed at Mullingar when Captain O'Carroll joined them in the middle of January, 1907. Two months later they sailed for Malta, which was reached on 22nd March of that year. After

serving for 1 year at Malta the regiment went on to India and were quartered at the Hill station of Dagshai from the middle of March, 1908, where they still remained on 17th July, 1909, the date of Captain O'Carroll's last return.

Inoculations.—The strength of the battalion on leaving Ireland for Malta was 485 and it was not brought up to its full strength until it proceeded to India in February, 1908, when it took over a large draft of 680 from the other battalion at Poona. During the time it was at Malta the strength averaged 450, in India it was brought up to 1,000 odd. The first inoculations were in part carried out before leaving Mullingar and continued during the stay of the regiment at Malta. In September, 1907, out of a strength of 450 then present, 300 had been inoculated; no fresh inoculations were done in Malta.

On the draft of 680 men being taken over in India from the other battalion, a small number of men were found to have been inoculated at other stations at various times, but no exact information could be obtained in some instances as the records had been mislaid. The number, however, was small and the majority of these men were shortly afterwards reinoculated. The inoculated strength was raised by the arrival of drafts from home who had been done before embarkation, and in June of 1908, a large number of reinoculations and fresh inoculations were carried out. The total number of inoculated present with the regiment on 17th July, 1909, was 611 out of a total strength of 1,006.

Incidence of enteric fever.—Two cases of para-typhoid fever occurred in uninoculated men at Malta, these have not been included in the return. Since the regiment has been in India there have been seven cases of enteric, two of them fatal, all of which occurred in uninoculated men. (NOTE.—The returns from this regiment included also two cases among inoculated men, but this was done under a misunderstanding since, on enquiry, it was found that these men had been inoculated at Poona and had been admitted to hospital there 8 months before the battalion to which Captain O'Carroll is attached reached India; they subsequently joined the 1st Battalion and were in error included in Captain O'Carroll's return.)

The latest return, dated from Dagshai, 17th July, 1909, gives the following results:—

Résumé.

Strength of the regiment	1,006
Number of inoculated	611
Number of uninoculated	395
Cases of enteric in inoculated	0
Deaths from enteric in inoculated	0
Cases of enteric in uninoculated	7
Deaths from enteric in uninoculated	2

Case incidence.

Inoculated	0 per 1,000
Uninoculated	17·5 "

Case mortality.

Inoculated	Nil.
Uninoculated	28·5 per cent.

Unit No. 9.

2nd Bn. Bedfordshire Regiment.

Medical Officers.—Captain C. M. Drew, R.A.M.C.; (later) Lieutenant J. H. Spencer, R.A.M.C.

History and movements of the regiment.—Lieutenant Drew joined the Bedfordshire Regiment at Tidworth, Salisbury Plain, on 29th July, 1907, and the regiment sailed for Gibraltar at the end of the following month. They reached this station on 2nd September, 1907.

There has been no change of station since then, and the regiment was still at Gibraltar on 26th June, 1909, the date of the latest report.

Lieutenant Drew was seconded for service with the Egyptian Army, and was relieved of his duties in connection with inoculation on 3rd June, 1908, by Lieutenant J. H. Spencer.

Inoculations.—A large number of inoculations were carried out by Lieutenant Drew before the regiment left England, and they arrived at Gibraltar with 294 inoculated men out of a strength of 793. Subsequent endeavours to increase the strength of the inoculated, both on the part of Lieutenant Drew and of Lieutenant Spencer, were not attended with much success. The total number of fresh inoculations carried out during the 1 year and 3 months under review is 76. The final return of the inoculated shows that there were 309 inoculated men present out of a total strength of 927.

The difficulty encountered by these officers in getting further volunteers for inoculation was in large part due to the fact that enteric fever is well known to be rare on Gibraltar.

Incidence of enteric.—In all, seven cases of enteric have occurred during the year and 3 months under review. One of these cases was fatal. It is worthy of record that in this unit the attempts to isolate the *Bacillus typhosus* during life were uniformly successful. In all seven cases the bacillus was isolated either from the blood or the stools, and identified during life. All of the cases were in non-inoculated men.

Résumé.

Strength of the regiment	927
Number of inoculated	309
Number of uninoculated	618
Cases of enteric in inoculated	0
Deaths from enteric in inoculated	0
Cases of enteric in uninoculated	7
Deaths from enteric in uninoculated	1

Case incidence.

Inoculated	0·0 per 1,000
Uninoculated	11·3 "

Case mortality.

Inoculated	Nil.
Uninoculated	14·2 per cent.

Unit No. 10.

13th Brigade, Royal Horse Artillery.

Medical Officer.—Captain A. S. Littlejohns, R.A.M.C.

History and movements of the unit.—Captain Littlejohns joined the brigade at Woolwich on 12th July, 1907. It was composed of "X" and "Y" Batteries, Royal Horse Artillery, and was under orders for South Africa. The brigade sailed in September, and arrived at their station, Roberts Heights, Pretoria, on 17th October, 1907. The brigade were still stationed at Roberts' Heights on 26th July, 1909, when the last return was sent by Captain Littlejohns.

Inoculations.—The strength of the two batteries when they sailed from England was 265, and of this number Captain Littlejohns was successful in inducing 224 men to volunteer for inoculation, 16 more being done on the voyage. All but 28 of these came up for their second inoculation. The full strength of the brigade was made up soon after reaching Africa, and fluctuated between 340 and 363. At the date of the last report the total strength was 354. 92 reinoculations were carried out during this time, but no fresh inoculations, and the latest strength of the inoculated was 255.

Incidence of enteric.—Up to the end of 1908, 14½ months after reaching South Africa, there had been four cases of enteric in the brigade with no deaths, three were in uninoculated men and one in an inoculated man. Four more cases occurred in 1909 bringing the total cases up to eight, two of them being in inoculated men. There have been no deaths.

Captain Littlejohns, speaking of the type of enteric encountered at Pretoria, says it is a mild one and that if a man has been previously inoculated there are practically no symptoms beyond the pyrexia.

This is certainly borne out by the description of the two cases which occurred in this brigade among the inoculated men. Each of these men had received two doses of vaccine. The first man's temperature fell to normal on the sixth day after admission and the second man's on the fifth day. In neither case could a bacillus be isolated and the diagnosis appears to have been based on the appearance of the patient. The agglutination in each case was no higher than would be accounted for by the previous inoculation, but Captain Littlejohns believed that they were genuine cases of enteric and, of course, they are recorded as such.

Captain Littlejohns himself suffered from a similar mild attack, the fever lasting 10 days, blood cultures were negative; he had been inoculated. His case is not, however, recorded as this return of the evidence in the units deals only with warrant officers, non-commissioned officers and men.

The frequency with which paratyphoid infections are encountered in Pretoria has been pointed out by Captain Statham, R.A.M.C.,[1] and must be borne in mind in any series of statistics dealing with inoculation in connection with troops stationed in this locality.

Résumé.

Strength of the unit	354
Number of inoculated	235
Number of uninoculated	119
Cases of enteric in inoculated	2
Deaths from enteric in inoculated	0
Cases of enteric in uninoculated	6
Deaths from enteric in uninoculated	0

Case incidence.

Inoculated	8·5 per 1,000
Uninoculated	50·4 „

Case mortality.

Inoculated	Nil.
Uninoculated	Nil.

UNIT No. 11.

1st Bn. Suffolk Regiment.

Medical Officer—Lieutenant J. B. G. Mulligan, R.A.M.C.

History and movements of the regiment.—Lieutenant Mulligan joined the Suffolk Regiment at Woolwich in September, 1907, they sailed for Malta in the middle of November and reached it on 27th November, 1907. The regiment was still quartered there on 17th June, 1909, the date of the latest return.

Inoculation.—Out of a strength of 890 men on arrival 367 men had been twice inoculated by Lieutenant Mulligan, as well as 24 who refused to have the second dose. No fresh inoculations were carried out during 1908, but in the beginning of 1909 36 fresh men were done and six men were re-inoculated. On 17th June, 1909, the total strength of the regiment was 926 and of these 412 had been inoculated.

Incidence of enteric.—There were no cases of enteric in this regiment during the 19 months they were stationed at Malta.

Résumé.

Strength of the regiment	926
Number of inoculated	412
Number of uninoculated	514

Case incidence and case mortality.

Nil.

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[1] Journal of the Royal Army Medical Corps, Vol. IX., page 226, 1907. *Ibid*, Vol. XI., page 617, 1908.

Unit No. 12.

1st (King's) Dragoon Guards.

Medical Officer—Lieutenant G. H. Stevenson, R.A.M.C.

History and movements of the regiment.—Lieutenant Stevenson joined this regiment at Hounslow on 1st September, 1907. It was under orders for India and sailed at the beginning of November, reaching Amballa, its first Indian station, on 2nd December, 1907. The regiment was still stationed there on 30th June, 1909, the date of the latest report.

Inoculations.—The strength of the regiment on leaving England was 527, and the number of men inoculated, either before sailing or on board the transport, was 220. After reaching India further inoculations were carried out on volunteers, and, by the end of April, 1908, out of a strength of 592 men, there were 450 inoculated; of these only five had refused the second dose.

At the date of the latest report there were 485 inoculated men present out of a strength of 620 on 30th June, 1909.

Incidence of enteric fever.—For the first 6 months of their Indian tour there were no cases of enteric in the Dragoon Guards. Commenting on this, Lieutenant Stevenson says: "Every regiment stationed here (in Amballa) has had some cases this year with the exception of the King's Dragoon Guards, who have so far escaped. . . . The King's Dragoon Guards is the only regiment in Amballa which can be regarded as an inoculated regiment."

Between June, 1908, and the end of that year, four cases occurred in the regiment, with one death; two of the cases were in inoculated men, and the others, one of which was fatal, in non-inoculated. One further case, in an inoculated man, occurred in the beginning of 1909, bringing the total number up to five on the 30th June, 1909.

Of the three cases which occurred in the inoculated, the first and the second were classed as "very mild," the third showed a severe onset but aborted early, a secondary rise of temperature of 4 days' duration being due to pleurisy. In the first case the Bacillus typhosus was isolated from the blood. The second was typical but blood culture failed. (In this case there had been an interval of 2½ months between first and second inoculations.) The blood culture was negative also in the case of the third man, and, from the agglutination results, Lieutenant Stevenson was inclined to consider it a paratyphoid infection; since, however, definite proof of this is lacking it has been included in the return as a true enteric case.

Lieutenant Stevenson himself suffered from an attack of continued fever diagnosed as and clinically typical of enteric fever, he had been inoculated against enteric 1 year and 7 months before. Blood cultures in his case were also negative, but his agglutinaton with the typhoid bacillus was negative on the various occasions on which it was tested while it reacted to paratyphoid "A" in a dilution of 1–80.

Résume.

Strength of the regiment	620
Number of inoculated	485
Number of uninoculated	135
Cases of enteric in inoculated	3
Deaths from enteric in inoculated	0
Cases of enteric in uninoculated	2
Deaths from enteric in uninoculated	1

Case incidence.

Inoculated	6·1 per 1,000
Uninoculated	14·8 "

Case mortality.

Inoculated	Nil.
Uninoculated	50 per cent.

Unit No. 13.

1st Bn. Lancashire Fusiliers.

Medical Officer—Lieutenant F. D. G. Howell, R.A.M.C.

History and movements of the regiment.—This regiment, at the time it was selected as one of the test units, was stationed at Malta but was under orders to proceed to India. Lieutenant Howell joined the battalion on board the transport at Malta at the end of November, 1907, and sailed with them to India. They reached their first station, Meerut, on the 16th December of that year. The regiment remained at Meerut for the remainder of the cold weather and then proceeded by route march to the hill station of Chakrata, which was reached on 31st March, 1908. They returned to Meerut on the 22nd December, 1908, once more leaving for Chakrata at the end of their second cold weather and arriving there on 30th March, 1909. On the date of Lieutenant Howell's last return, 2nd July, 1909, the regiment was still at this latter station.

Inoculations.—As Lieutenant Howell was not to join the battalion until their departure for India, the inoculations were arranged for at Malta, and thanks chiefly to the energy of Captain Winder, R.A.M.C., and the support of the officers of the regiment, a very large proportion of volunteers was obtained. The strength of the regiment on leaving Malta was 830, and of this number 716 had been inoculated. A few of the second inoculations which remained to be done were performed on board the transport by Lieutenant Howell. On completing their full strength in India, further inoculations were done, and on 5th May, 1908, out of 940 men present with the regiment, 796 had been inoculated, a percentage of 84. The proportion was raised still higher during this year, and on 4th January, 1909, out of 997 men no less than 927 had been inoculated, equal to 92·8 per cent.

The last report, dated 2nd July, 1909, the strength present was 959 and of these 890 had been inoculated, precisely the same percentage as above, viz., 92·8 per cent.

Incidence of enteric.—This being the most thoroughly inoculated regiment of the 24, it is naturally of interest to see how far it has suffered from enteric during the 1 year and 7 months during which it had been in India. Only two mild cases have occurred, and although both of these were in men who had been inoculated, this is hardly surprising when the fact that 92·8 per cent. of the regiment was inoculated is borne in mind. These two cases were admitted to hospital respectively 1 year and 4 months and 1 year and 5 months after the arrival of the regiment in India, therefore, for this period of 1 year and 4 months there had not been a single case of enteric in the battalion. That this fact can hardly be accounted for by absence of any danger of infection seems clear from the following, firstly, the fact that it was twice stationed at Meerut for several months at a time, and the danger of enteric here may be realized by referring to the history of the 17th Lancers, who were also stationed at Meerut at the same time. Secondly, in their first march up to Chakrata, Lieutenant Howell mentioned that, although the Fusiliers had no cases, the Cameronians, who did the same march the year before, had 10 or 12 cases contracted on the journey. Thirdly, when the regiment was out at manœuvres at the end of 1908, it had no cases, while the South Lancashire Regiment which was brigaded with it had six.

It would appear, therefore, in spite of the incidence having been confined to the inoculated, that the Lancashire Fusiliers had come well out of the test.

Résumé.

Strength of the unit	959
Number of inoculated	890
Number of uninoculated	69
Cases of enteric in inoculated	2
Deaths from enteric in inoculated	0
Cases of enteric in uninoculated	0
Deaths from enteric in uninoculated	0

Case incidence.

Inoculated	2·2 per 1,000
Uninoculated	Nil.

Case mortality.

Inoculated	Nil.
Uninoculated	Nil.

Unit No. 14.

3rd Bn. Worcestershire Regiment.

Medical Officer—Lieutenant W. H. Forsyth, R.A.M.C.

History and movements of the regiment.—The 3rd Bn. Worcestershire Regiment was stationed at Aldershot when Lieutenant Forsyth joined them on the 2nd September, 1907. They sailed for South Africa at the end of November and reached Wynberg on 19th December of that year. A detachment of 135 non-commissioned officers and men went to Standerton on Mounted Infantry duty and these rejoined the Headquarters of the regiment on 10th June, 1908, at Wynberg.

On the reduction of the strength of the garrison in South Africa the Worcestershire Regiment was one of those concerned and was ordered to return to England. They left Wynberg for England on 7th September, 1908, and since then they have been stationed at Dover. The period, therefore, for which they were under observation in connection with inoculation was only 9 months and ceased with their departure from South Africa on the date mentioned.

Inoculations.—Although Lieutenant Forsyth carried out over 200 inoculations before the regiment sailed, he found considerable difficulty in getting so many men to come forward for a reason which is of sufficient interest to detail. It was known in the regiment that one of their own officers had recently been inoculated by his family doctor and had been very seriously ill in consequence. Lieutenant Forsyth therefore found it very hard to convince either officers or men of the simple nature of the procedure and of the moderate severity of the reactions which might be expected. It so happens that the history of this case is known to Colonel Leishman who was called in consultation to see it. He found that the medical man had, in all good faith, given this officer two large doses of anti-typhoid *serum*, obtained from a commercial firm, under the impression that this was typhoid "vaccine." Not unnaturally, the patient had severe symptoms of anaphylaxis the result of two large doses of horse serum at an interval of 10 days.

The regiment arrived in South Africa with 206 inoculated men out of a strength of 614. Only four fresh inoculations were carried out in Africa before the regiment returned home, at which time the inoculated stood at 209 out of 633.

Incidence of enteric.—Only three cases occurred in the regiment during the 9 months; the first of these developed on the 18th day of the voyage out, so was probably contracted in England, the two others were admitted in February, 1908, none of them were very severe and all recovered. In one of the latter cases there was suspicion of paratyphoid on account of the agglutination results, but blood cultures failed to verify the diagnosis since no bacteria were isolated on the bile-salt media. None of the cases had been inoculated.

Résumé.

Strength of the unit	633
Number of inoculated	209
Number of uninoculated	424
Cases of enteric in inoculated	0
Deaths from enteric in inoculated	0
Cases of enteric in uninoculated	3
Deaths from enteric in uninoculated	0

Case incidence.

Inoculated	Nil.
Uninoculated	7 per 1,000

Case mortality.

Inoculated	Nil.
Uninoculated	Nil.

Unit No. 15.

1st Bn. Yorkshire Regiment.

Medical Officer—Captain S. O'Grady, R.A.M.C.

History and movements of the regiment.—Captain O'Grady joined this regiment at Aldershot on 3rd September, 1907, and sailed with them to Egypt in the middle of January, 1908. The headquarters and seven companies arrived at Alexandria on 30th

January, 1908, and one company at Cyprus on the 29th of the same month. Captain O'Grady was himself stationed at Alexandria during the 18 months under consideration, but he kept careful touch of all that occurred in the company detached to Cyprus, and the details given below refer, as usual, to the whole of the regiment. The regiment moved from Alexandria to Cairo on 29th January, 1909, and was still there on the date of the last report, viz., 29th July, 1909.

Inoculations.—On leaving Aldershot the strength of the regiment was 794, and of these 439 were inoculated, either there or on board the transport. During the stay of the regiment in Egypt, 38 fresh inoculations were carried out, chiefly on men of the drafts which joined from England, and the latest figures show 460 men inoculated out of a strength present on 29th July, 1909, of 847.

Incidence of enteric.—This has been slight, only three cases having occurred in the battalion during the 18 months it has been under observation. Two of these cases, one of them fatal, occurred in 1908, the third in 1909. None of them were in inoculated men.

Résumé.

Strength of the unit..	847
Number of inoculated	460
Number of uninoculated	387
Cases of enteric in inoculated	Nil.
Deaths from enteric in inoculated	Nil.
Cases of enteric in uninoculated	3
Deaths from enteric in uninoculated	1

Case incidence.

Inoculated	Nil.
Uninoculated	7·7 per 1,000

Case mortality.

Inoculated	Nil.
Uninoculated	33·3 per cent.

Unit No. 16.

3rd Bn. King's Royal Rifle Corps.

Medical Officer—Lieutenant R. W. D. Leslie, R.A.M.C.

History and movements of the regiment.—Lieutenant Leslie joined the regiment at Aldershot on the 23rd of December, 1907, and sailed with them for Crete in the beginning of February, 1908. The headquarters and 5½ companies arrived at Candia on 25th February, 1908, half a company was detached to Canea, Crete, and two companies to Malta, where they arrived on the 28th of February, 1908.

Two of the companies arrived at Malta from Candia on 30th July, 1908, and the remaining companies on 22nd January, 1909, the regiment being subsequently united at Malta, whence the last report was sent off on 22nd June, 1909.

Inoculations.—The regiment left Aldershot 889 strong, and of these 177 had been inoculated. During their stay at Crete and Malta, 109 fresh inoculations were carried out, and on 22nd June, 1909, the date of the last return from Malta, there were 217 inoculated men present with the battalion out of a strength of 928.

Incidence of enteric.—No cases have occurred during the period of 1 year 4 months, during which the regiment has been under observation.

Résumé.

Strength of the unit..	928
Number of inoculated	217
Number of uninoculated	711

Case incidence and case mortality.

Nil.

Unit No. 17.

7th Dragoon Guards.

Medical Officer—Lieutenant E. Gibbon, R.A.M.C.

History and movements of the regiment.—Lieutenant Gibbon joined this regiment at Canterbury on 1st August, 1908. They sailed from England in the middle of the following month and reached Cairo on 27th September, 1908. Here they were quartered at Abassieh where the regiment remained while under observation.

Inoculations.—171 men were inoculated out of a strength of 575 before or during the voyage to Egypt, and since then Lieutenant Gibbon has carried out 79 fresh inoculations. The latest figures, dated 25th June, 1909, show 242 inoculated men present out of a strength of 561.

It may be noted that the reaction after one of these inoculations was of exceptional severity, one of the men after his second dose becoming collapsed and having to be taken to hospital suffering from diarrhœa and vomiting. He denied having been to the canteen, but as his vomit smelt strongly of beer there can be little doubt that this was an instance of the bad effect of alcohol when taken shortly after inoculation. He recovered completely in a couple of days.

Incidence of enteric.—During the 9 months which the regiment was under observation there was only one case of enteric fever, ending fatally; this was in an uninoculated man.

Résumé.

Strength of the regiment	561
Number of inoculated	242
Number of uninoculated	319
Cases of enteric in inoculated	Nil
Deaths from enteric in inoculated	Nil.
Cases of enteric in uninoculated	1
Deaths from enteric in uninoculated	1

Case incidence.

Inoculated	Nil.
Uninoculated	3·1 per 1,000

Case mortality.

Inoculated	Nil.
Uninoculated	100 per cent.

Unit No. 18.

6th Inniskilling Dragoons.

Medical Officer—Lieutenant J. du P. Langrishe, R.A.M.C.

History and movements of the regiment.—The inoculation history of this regiment starts from the date of their arrival at Mhow in India. Before this they had been stationed in Egypt, and Lieutenant Langrishe joined them on board the transport at Suez on 29th September, 1908. While at Cairo the regiment had been very well inoculated, over 90 per cent. of the strength, and on enquiry it was found that they had not had a case of enteric for a year before Lieutenant Langrishe joined them. The date of their arrival at Mhow was 9th October, 1908, and the latest report was dated from the same station on 9th July, 1909.

Inoculations.—Owing to the large number of inoculations which had been done in Egypt little remained for Lieutenant Langrishe to do in this respect; when he first joined the regiment, out of a strength of 433, 397 were already inoculated; later, as fresh drafts joined the regiment in India, fresh inoculations were carried out, and on the 9th July, 1909, out of a strength then present of 605 the inoculations numbered 547.

Incidence of enteric.—No cases had occurred during the 9 months under consideration.

Résumé.

Strength of the unit	605
Number of inoculated	547
Number of uninoculated	58

Case incidence and case mortality.

Nil.

Unit No. 19.

1st Bn. Border Regiment.

Medical Officer—Lieutenant B. Johnson, R.A.M.C.

History and movements of the regiment.—Lieutenant Johnson joined the Border Regiment at Gibraltar, where they had been stationed for some time, on 4th October, 1908, the date on which they sailed for India. The regiment arrived at Wellington in Madras on 31st October, 1908, and they have had no change of station since that time.

Inoculations.—A certain number had been carried out at Gibraltar before the regiment sailed and others were performed by Lieutenant Johnson on board the transport so that, on arrival at Wellington, there were 301 men inoculated out of a strength of 999. Since they have been in India 121 further inoculations have been carried out and on 14th July, the date of the last return, there were 422 inoculated men out of a total strength of 990.

Incidence of enteric.—There has only been one case so far in this regiment, terminating in recovery; it was in a man who had not been inoculated.

Résumé.

Strength of the regiment	990
Number of inoculated	422
Number of uninoculated	568
Cases of enteric in inoculated	Nil.
Cases of enteric in uninoculated	1
Deaths from enteric in uninoculated	0

Case incidence.

Inoculated	Nil.
Uninoculated	1·7 per 1000

Case mortality.

Inoculated	Nil.
Uninoculated	Nil.

Unit No. 20.

2nd Bn. Somersetshire Light Infantry.

Medical Officer—Lieutenant H. G. Gibson, R.A.M.C.

History and movements of the regiment.—Lieutenant Gibson joined this regiment at Plymouth on 8th September, 1908. They sailed for Malta at the beginning of November and reached this station on 12th November, 1908. There has been no change of station since then.

Inoculations.—On leaving England there were only 486 men present with the battalion as they were to make up their full strength at Malta from their other battalion. Out of this number of 486 Lieutenant Gibson inoculated 283. Since they have been stationed at Malta he only did six more inoculations and on the date of the latest report, 20th June, 1909, there were 285 inoculated men present out of a strength of 890. It has been found difficult to persuade men to be inoculated in a station in which there

is known to be but little enteric fever unless such a regiment is shortly changing its station to one less healthy. This point has already been alluded to in connection with some of the other test units.

Incidence of enteric.—In spite of the supposed freedom from enteric five cases have occurred in the regiment during the 7 months of their stay in Malta. One of these proved fatal. None were in inoculated men. In addition to these five cases there were two others, also uninoculated, in whose cases it was possible to demonstrate by blood-culture that the disease was paratyphoid. Neither of these cases have been included in the returns of this unit.

An incidental point of interest is that Lieutenant Gibson discovered a typhoid-carrier among the men who joined the regiment from the 1st Battalion from India, it appeared possible that some of the five cases may have contracted their infection from this carrier.

Return.

Strength of the unit	890
Number of inoculated	285
Number of uninoculated	605
Cases of enteric in inoculated	Nil.
Deaths from enteric in inoculated	Nil.
Cases of enteric in uninoculated	5
Deaths from enteric in uninoculated	1

Case incidence.

Inoculated	Nil.
Uninoculated	8·2 per 1,000

Case mortality.

Inoculated	Nil.
Uninoculated	20 per cent.

Unit No. 21.

1st Bn. Worcestershire Regiment.

Medical Officer.—Lieutenant J. L. Wood, R.A.M.C.

History and movements of the regiment.—This battalion was stationed at Malta at the time Lieutenant Wood was posted to it, it was under orders for India and Lieutenant Wood joined them on the eve of their departure on 11th November, 1908. They reached Bareilly on 28th November, 1908, and had no change of station during the 8 months of observation.

Inoculations.—The battalion had been well inoculated at Malta by other medical officers before they were joined by Lieutenant Wood, the numbers being 601 men inoculated out of a strength of 893. Since their arrival Lieutenant Wood carried out a very considerable number of fresh inoculations on those who formerly declined and on fresh arrivals to the battalion, in all 311 fresh inoculations and 37 re-inoculations. At the date of his last report, 9th July, 1909, there were 912 inoculated men present out of a total strength of 1,001. This gives a percentage of inoculated equal to 90 per cent. of the whole strength.

Incidence of enteric.—In the case of this regiment, as happened in Unit No. 2, the 17th Lancers, there was a considerable incidence of the disease within a short time of the regiment reaching their first Indian station, 15 cases and three deaths having occurred within the 8 months during which they were under observation. They present a further likeness to the case of the 17th Lancers, inasmuch as the inoculations of the men were of recent date. In view of the very high percentage of inoculated men the effects of inoculation upon the incidence of the disease are of exceptional interest.

The first case was probably contracted before the regiment left Malta as he went sick on the voyage and had to be left in hospital at Bombay. The two next occurred in January, and the remainder at various intervals up to the middle of July.

As regards inoculation, four cases occurred in inoculated men, all of whom recovered, the remaining 11 cases were in uninoculated men; of these three proved fatal.

Of the four cases in the inoculated, two were classed as "very mild," the duration of the pyrexia being 10 and 6 days respectively (the typhoid bacillus was isolated from the blood in the latter); one was moderately severe, but without complications, and the fourth, which was a mild attack lasting 12 days, was a case of para-typhoid as this bacillus was isolated from the blood during life. The case is not, however, excluded in this instance, since it is possible that the cases among the uninoculated may also have included some of para-typhoid, Lieutenant Wood having been ordered away from the station for a month, and being, therefore, unable to make the necessary tests in the cases which occurred in his absence.

A further case, which ended fatally, is mentioned by Lieutenant Wood, although not included in his return, that of a man who was inoculated in the incubation period of the disease, his illness commencing 6 days after his first dose. As in the case mentioned in connection with Unit No. 6, this case is also excluded from the *non*-inoculated list.

The résumé of the results in this regiment shows a very striking contrast between the incidence of the disease in the inoculated and in the very small group of men who refused inoculation in spite of frequent advice.

Résumé.

Strength of the regiment	1,004
Number of inoculated	912
Number of uninoculated	92
Cases of enteric in inoculated	4
Deaths from enteric in inoculated	Nil.
Cases of enteric in uninoculated	11
Deaths from enteric in uninoculated	3

Case incidence.

Inoculated	4·3 per 1,000
Uninoculated	119·5 „

Case mortality.

Inoculated	Nil.
Uninoculated	27·2 per cent.

Unit No. 22.

2nd Bn. Liverpool Regiment.

Medical Officer—Lieutenant W. Mitchell, R.A.M.C.

History and movements of the regiment.—Lieutenant Mitchell joined this regiment at Gosport on 13th November, 1908; it sailed for India in the middle of the following month and arrived at Amballa on 8th January, 1909. At the end of the cold weather the battalion moved to Sabathu, in the hills, reaching this station on 30th March, 1909; there was no further change of station up to the date of the latest report, 8th July, 1909. The battalion, however, while at Sabathu furnished a number of detachments to smaller stations, the following being the distribution in July, 1909:—Headquarters and six companies at Sabathu itself, one company at Jutogh, one company at Dagshai, and smaller detachments at the following: Nasirabad, Kalka, Kasauli, Simla and Amballa. The figures below refer, as usual, to the whole of the regiment, and include all the men detached to these various stations.

Inoculations.—On leaving Gosport, the strength of the 2nd Battalion was 461, and of these 405 were inoculated by Lieutenant Mitchell before embarkation. The battalion took over a large draft from the 1st Battalion on arrival at Karachi, bringing the strength of the regiment up to 900, and of these a certain number had already been inoculated so that, with the addition of a few men inoculated on the voyage, the total number of the inoculated on arrival at Amballa was 471, out of a strength of 900. Since then a few fresh inoculations, 52 in all, were carried out, and at the date of the latest report, 8th July, 1909, there were 510 inoculated men present out of a strength of 909.

Incidence of enteric.—Two cases of enteric fever have occurred in the regiment during the 6 months of observation. They were admitted to hospital at Sabathu on 11th and 12th May, respectively, and came from the same barrack-room. In his first report upon these cases Lieutenant Mitchell stated that one of the men had been inoculated but not the other. In a later letter, however, he said that this was an error, and that the second man also was found to have had a single dose of vaccine 18 months before.

In both these cases there appear to have been considerable doubts as to the accuracy of the diagnosis. The first, Private C., received two doses of vaccine and was admitted to hospital in one month from the date of the last inoculation, it is therefore possible, supposing it to have been a true attack of enteric, that he was inoculated within the incubation period, since the latest authorities place the outside limit of this period at 45 days. However that may be, Lieutenant Mitchell states: "The bacteriological results are all negative, and I am of opinion the case is probably not one of enteric." The temperature chart is, however, suspicious, and he is recorded as having had a true attack of enteric.

The second case, Private H., was the man who had received only a single dose of vaccine 18 months before and the diagnosis here is even more doubtful than in the former case. The attack commenced as one of diphtheria, the diphtheria bacillus being isolated from his throat and anti-toxin treatment employed; when apparently convalescing from this his temperature again rose and he was diagnosed enteric fever, repeated bacteriological investigation of the stools failed, however, to demonstrate the presence of the typhoid bacillus and his blood serum gave twice as strong an agglutination reaction with para-typhoid "B" as with B. typhosus. Lieutenant Mitchell states "The case is probably one of para-typhoid."

In this case also, since the official diagnosis remained as enteric the case is allowed to stand, although it seems unfair to inoculation to include either in the statistics.

It may be added in conclusion that the single dose of vaccine given to Private H. in 1907 was 7 months old at the time and, therefore, probably inactive so that, supposing it to have been a true attack, he can have had little or no protection left as the result of this inoculation 18 months before.

Résumé.

Strength of the regiment	909
Number of inoculated	510
Number of uninoculated	399
Cases of enteric in inoculated	2
Deaths from enteric in inoculated	0
Cases of enteric in uninoculated	0
Deaths from enteric in uninoculated	0

Case incidence.

Inoculated	3·9 per 1,000
Uninoculated	Nil.

Case mortality.

Inoculated	Nil.
Uninoculated	Nil.

UNIT No. 23.

2nd Bn. Devonshire Regiment.

Medical Officer—Lieutenant W. K. Beaman, R.A.M.C.

History and movements of the regiment.—Lieutenant Beaman joined this regiment at Devonport on 12th September, 1908. They sailed from England at the beginning of January, 1909, calling first at Malta, where three companies were left on 16th January, and then going on to Crete where the headquarters and five companies arrived on 19th January, 1909. This was still the distribution of the battalion on 20th June, the date of the last report.

Inoculation.—On leaving Devonport the strength of the regiment was 481 and of these Lieutenant Beaman had inoculated 253. On the regiment completing their full strength from the linked battalion there does not appear to have been any addition to the numbers of the inoculated and on the date of the latest return, 20th June, 1909, there were still only 253 inoculated men out of a total strength of the whole regiment of 905. No fresh inoculations were carried out either at Crete or Malta.

Incidence of enteric fever.—Only one case of enteric occurred in the regiment, a man of the detachment at Malta, diagnosis being confirmed by the isolation of the typhoid bacillus from the blood and stools. It was severe but the man recovered. He had not been inoculated.

Résumé.

Strength of the regiment	905
Number of inoculated	253
Number of uninoculated	652
Cases of enteric in inoculated	Nil.
Deaths from enteric in inoculated	Nil.
Cases of enteric in uninoculated	1
Deaths from enteric in uninoculated	Nil.

Case incidence.

Inoculated	Nil.
Uninoculated	1·5 per 1,000

Case mortality.

Inoculated	Nil.
Uninoculated	Nil.

Unit No. 24.

1*st Bn. Royal Scots.*

Medical Officer—Lieutenant A. C. Amy, R.A.M.C.

History and movements of the regiment.—Lieutenant Amy joined the Royal Scots at Shorncliffe on 12th October, 1908, and sailed with them at the end of January to India. The regiment reached Ranikhet on 18th February, 1909, and have had no change of station up to the date of the last return on 30th June, 1909.

Inoculations.—The strength of the battalion on leaving England was 542. Lieutenant Amy's difficulties were even greater than is usual, but he overcame them successfully, and was able to get 476 men inoculated before the regiment sailed. The battalion completed its full strength by a large draft from the 2nd Battalion at Bombay, and the strength on reaching Ranikhet were 910, of whom the inoculated numbered 561, the additions being due to further inoculations carried out on board the transport, and to the small number of men in the draft of the 2nd Battalion who had previously been inoculated.

Since the regiment has been stationed at Ranikhet, Lieutenant Amy has carried out a number of fresh inoculations, which brought the total strength of the inoculated on 30th June, 1909, up to 702, out of a strength then present of 881.

Incidence of enteric.—Up to the date of the last report, 30th June, 1909, there had been three cases of enteric in the regiment. The first two were in uninoculated men, both recovered though one had a relapse, the third was in an inoculated man who had received two doses of vaccine 23 months before, the case was very mild and there was some doubt as to the diagnosis, no definite information was obtained from agglutination or from cultures, the latter failing. The case was admitted on 6th July and was making a good recovery at the time of the report.

Lieutenant Amy also mentions a case of fairly severe and typical enteric in a child aged 6, the son of one of the Warrant Officers of the regiment, as interesting on account of this child being the only uninoculated member of a family of four.

Résumé.

Strength of the regiment	881
Number of inoculated	702
Number of uninoculated	179
Cases of enteric in inoculated	1
Deaths from enteric in inoculated	Nil.
Cases of enteric in uninoculated	2
Deaths from enteric in uninoculated	Nil.

Case incidence.

Inoculated	1·4 per 1,000
Uninoculated	11·1 „

Case mortality.

Inoculated	Nil.
Uninoculated	Nil.

CHAPTER II.

Summary of the Statistical Results.

(With Table I.)

The table which is appended to this chapter summarizes the results recorded in the case of the 24 units, whose detailed history has just been given. These results deal with the inoculation history of each unit from the date when it first came under observation until the month of July, 1909. It is regretted that for various reasons, including the temporary disablement of Sir William Leishman by illness, it has not been found possible to bring the figures up to a more recent date, but, at the same time, the table gives on the whole as good a view of the effects of inoculation as could be expected under the conditions which obtain in service abroad. Subsequent to the date at which the records terminate—July, 1909—the military authorities became, in ever-increasing degree, more sympathetic towards inoculation, and the soldiers themselves more and more convinced of its efficacy in limiting the risks of infection. Consequently, increasing numbers presented themselves for inoculation with the result that, in many units, the very large majority of men were inoculated, and but a few were left as uninoculated controls. Indeed, in the case of some of these "Test-Units," the latest figures have shown that 100 per cent. are inoculated. This being so, such units had obviously become useless for any comparative statistical test of the protective value of inoculation. On the other hand, at the time the table was drawn up, the two groups of inoculated and non-inoculated were fairly homogeneous, there being **10,378** of the former and **8,936** of the latter class, out of a total of **19,314** men. The subsequent disproportion of the two classes, when realized by the War Office authorities, led to the termination of the system of attaching special medical officers to these units and to the substitution of the customary official mechanism for the collection of statistics bearing upon the results of inoculation.

The present table, then, appears to furnish as good an illustration of the effects of inoculation as could be hoped for, in view of the homogeneity of the material and the great care which has been exercised in the verification of the diagnosis, as will be explained below.

As will be seen, Table I. summarizes the information detailed in the preceding chapter in connection with each individual unit, but a few words of further explanation are called for that it may be fully understood. These remarks may conveniently be given under the headings of the various columns which compose the table.

Columns 1 and 2.—The units are numbered consecutively in the order in which they went on foreign service or came under the charge of a special medical officer.

Column 3.—Gives the name of the special medical officer attached to each unit. In two cases—units 5 and 9—the original medical officer was detached from the unit

and his place was taken by another who stayed with the unit till the date of the last return. In most instances the medical officer named was with his unit for the whole of the time mentioned except for short absences due to leave or to sickness, during which his duties were carried out by another officer whom he had thoroughly posted in the special work.

Column 4.—In this is shown the station at which the unit was serving at the date of the last report of the medical officer. Naturally, in the case of the units which had been longest under observation, there have been one or more changes of station during the period of observation; where this occurred full details will be found under the history of the special unit in the foregoing chapter.

Column 5.—Date of arrival at first foreign station. This calls for no comment except that in a few instances the unit did not start from home but from one of the Mediterranean stations. These, too, are notified in the preceding chapter.

Columns 6 and 7.—Give the date of the last return of the medical officer and the total period under observation in years and months. This period will be seen to range from 3 years and 10 months in the case of the 17th Lancers, to 4 months in the case of the 1st Bn. Royal Scots. The average period during which the 24 units were under observation works out at 1 year and 8 months.

Columns 8, 9 and 10.—These give the total strength of the particular unit, and it has been thought well to give three separate figures in connection with this for the following reasons :—

The population of a regiment is, to a considerable extent, a floating one, it is continually sending men to the Reserve and making up this drain by drafts of recruits, while smaller losses of strength occur through invaliding, death, transfer to other corps and desertion. Again, a regiment on proceeding from a home station to a foreign one very frequently sails very much under its full strength and only completes its establishment on arrival abroad from men belonging to its linked battalion which is probably on the point of returning home and may transfer a large proportion of the men who have still some time to serve to the sister battalion.

In these ways there is, not uncommonly, a very marked difference between the strength of the regiment on its arrival at its first foreign station and on its being made up to its full strength at a later period. Once it has been made up to its establishment further changes are small, except during what is known as the trooping season when time-expired men are sent home and when drafts of recruits come out to take their place.

It might have been possible to present the figures in connection with total strength in the form of averages, but this would have been difficult to calculate and of very doubtful benefit as regards clearness so the alternative has been adopted of showing the total strength of each unit under three heads :—1st. The actual strength on the date at which the medical officer joined his unit. 2nd. The actual strength when the unit had arrived at its first foreign station. 3rd. The actual strength of the unit on the date at which the medical officer made his last return.

The best impression of the average range of this fluctuation is to be obtained by contrasting the strength given in columns 9 and 10. Further information upon these fluctuations is given in the history of the individual units.

It must be borne in mind that the numbers given are those of warrant officers, non-commissioned officers and men only; officers have not been included, neither have women and children.

Turning now to the totals of the 24 units it will be seen that when the respective medical officers joined their units the total strength was **13,980**. When their units had reached their first foreign stations and had made up their full strength this number had increased to **18,483**, while on the dates on which the last returns were made up the total figure stood at **19,314**. It so happens that the last total is higher by about 800 men than the total in column 9, which records the figure when the units were first made up to their full strength, but this difference between the two totals does not greatly affect the results since the fluctuations due to the causes already mentioned are constantly operative and, when concerned with such a large body of men, would be found to amount to something approximating this difference of 800 at separate summations made at intervals of a few months.

Columns 11, 12, 13 and 14 deal with the totals of the men inoculated in each unit, and here it has been thought well to give two series of figures, the first showing the number of inoculated men present on arrival at the first foreign station, the second, the total of inoculated men present with the unit on the date of the last return. It will

be seen that, as might be expected, the numbers in almost every instance show a marked increase, the degree of this increase bearing testimony to the success of the attached medical officer in getting the men to come forward for inoculation. Naturally, this was found to be an easier task when the unit in question was actually serving in a station where enteric fever was present and where the men had the dangers and results of the infection before their eyes. As will be seen, the total number of the inoculated rose from 6,815 to 10,378 at the date of the latest return. It is on the last figure that the case-incidence per 1,000 has been calculated and, although the difference between the two totals is, in this instance considerable, the latter figure may be taken as approximately representing the strength of the inoculated throughout the period under observation, since the great rise in the number of the inoculated in most of the units occurred very shortly after their arrival at their foreign stations.

Columns 13 and 14 record respectively the total number of cases of enteric fever and deaths from this disease which have occurred among the inoculated men in each unit during the whole period under observation.

A few words must be said here to emphasize the fact of the very special care which has been taken in the diagnosis of enteric fever by the medical officers of each unit. Owing to the system adopted it may safely be said that no case of enteric occurring in the various units can possibly have escaped observation, since every doubtful case of continued fever was specially investigated by all the modern methods which were available; these included, in all cases, the agglutination reaction, and in many, if not in the majority, the attempted isolation and identification of the bacillus, either from the blood or stools during life, or from the body after death. Owing to the exigencies of service conditions, which, as may be readily guessed, were not infrequent, it was not possible to carry out the blood cultures in every instance, but whenever this was possible it was attempted and the success which was obtained was considerable, especially in view of the fact that the recent great improvements in the technique of this operation were not then available. In this connection attention may also be called to Chapter IV. of this part of the Report where will be found the conclusions of the sub-committee who carefully studied the detailed history and the temperature charts of all the cases which have been included in this Table, both in the inoculated and in the non-inoculated men. As will be seen, this Sub-Committee had no doubt as to the correctness of the diagnosis in all of these cases.

Another point which must be mentioned is the question of paratyphoid fever, as it has an important bearing on this Table and on any conclusions which may be drawn from it as to the protective value of typhoid inoculation. It is only since the earlier units came under observation that the frequency of paratyphoid infections in India and South Africa has been brought to notice by Army Medical officers, thanks to the increasing frequency of blood cultures and the systematic bacteriological investigation to which every case of continued fever in a soldier is now subjected. This question, therefore, became one of increasing importance in connection with the returns from the units the longer they were abroad, and a decision had to be arrived at as to how such cases should be dealt with in completing the table of the incidence of enteric. It would have been manifestly unfair to include cases of paratyphoid fever occurring among men inoculated with typhoid vaccine as this would tell against inoculation unjustly, for the reason that, as has been shown in Part I. of the Report, experiment has proved that typhoid inoculation gives rise to little or no development of substances protective against a paratyphoid infection. On the other hand, if the diagnosis of paratyphoid was too readily accepted, some danger would exist of excluding cases of genuine typhoid from the table.

After careful consideration it was decided to admit the diagnosis of paratyphoid fever only when paratyphoid bacilli has been isolated from the patient and identified by cultural tests. Cases, especially in the earlier units, were not infrequently recorded by the medical officers as paratyphoid fever, the diagnosis having been made on the agglutination reaction and, to a smaller extent, on the clinical signs. Such diagnoses it was thought inadvisable to accept in view of the notorious difficulty of interpreting correctly the "group agglutinations" which are so frequently met with in fevers due to the typho-coli group of bacteria. Doubtful cases, then, of paratyphoid fever, in which the bacillus had *not* been isolated and identified have in all cases been included in this Table as cases of enteric fever. It is possible, and indeed most probable, that by this system certain cases of paratyphoid have been included which ought not to have been, but it was felt better to run this risk than to adopt the alternative of excluding cases because they were possibly paratyphoids; the latter procedure might justly have been criticized as tending towards an unduly favourable view of the effects of typhoid vaccine.

It is hardly necessary to add that the same strict rule was applied to cases of paratyphoid or suspected paratyphoid occurring among the other group of non-inoculated men and such cases were only excluded from the returns when paratyphoid bacilli had been isolated and identified; otherwise they have all been included as cases of genuine enteric fever. In this way, although as has been said, some cases that were really paratyphoid may be included in one or both groups, the two groups are at least comparable since each has been submitted to the same arbitrary method of elimination.

As regards the totals recorded at the foot of the columns, it will be seen that there have been **56** cases of enteric fever among the men of the 24 units during the period for which they have, respectively, been under observation, and that of these 56 cases **5** have died. Full details with regard to the inoculations will be found in Table II. and an analysis of this Table is given in Chapter III., so no more need be said about them in this place.

Columns 15, 16, 17 and 18.—These deal in similar fashion to the preceding four columns with the strength of the *non*-inoculated in the various units and with the incidence and mortality of enteric in this group.

The number of the non-inoculated in each unit and at each period has been arrived at by subtracting the number of the inoculated men on a certain date from the total number of men present with the unit on that date. As will be seen, in this instance the strength of the group is a falling strength and the total number of non-inoculated fell from **11,668**, when the units reached their first foreign station, to **8,936**, the strength which was present with the units on the dates at which the latest returns were made up.

Columns 17 and 18 show the actual number of cases of and deaths from enteric fever which occurred in this non-inoculated group during the whole of the period during which the unit had been under the observation of the attached medical officer.

The remarks which have been made upon the cases and deaths from enteric occurring among the inoculated group apply equally to this non-inoculated group. The same pains have been taken, in every instance, to verify the diagnosis in every possible manner, to exclude none but *bonâ fide* cases of paratyphoid fever, and to make certain that no case escaped observation and record through a man going sick at some out-station where he had been detached on some special duty.

The Sub-Committee already referred to submitted the temperature charts and detailed medical history of these cases to the same careful scrutiny as those of the inoculated men, and their report may be studied in connection with the correctness of the diagnosis. (*See* Chapter IV., Part II., page 98).

Looking to the totals at the foot of columns 17 and 18 it appears that in the group of **8,936** non-inoculated men present with the 24 units on the date of the last returns **272** had contracted enteric fever during the period under observation and that **46** of these cases were fatal.

Columns 19 and 20 record the case-incidence per 1,000 men in each of the groups of inoculated and non-inoculated. The strengths from which these incidences have been derived are those which were present in each unit on the date on which the last return was completed and will be found in columns 12 and 16, respectively. In considering these incidences it must be borne in mind that they refer to the incidence calculated for the whole period during which the unit in question has been under observation and that this ranges from 3 years and 10 months, in the case of the 17th Lancers, to 4 months in the case of the 1st Bn. Royal Scots. The average period of observation of the 24 units was 1 year and 8 months.

It will be seen at once that the incidence is in favour of inoculation in every instance except two, units Nos. 13 and 22, but in each of these regiments there had only been two cases of enteric during the whole period of observation so the figures are not of much significance.

The case-incidence of the whole body of *inoculated* men is **5·39** per **1,000**, while that of the *non-inoculated* group is **30·4** per **1,000**.

The "probable error" of these figures has been worked out by Colonel R. G. S. Simpson, C.M.G., R.A.M.C., who finds that in the case of the incidence of 5·39 among the inoculated the probable error is $\pm$ 0·48, while, in the case of the incidence of 30·4 among the non-inoculated, the probable error is $\pm$ 1·23. Colonel Simpson concludes from his examination of the figures and the smallness of the probable errors that the difference in the incidence of enteric in the two groups is, in statistical language, "significant."[1]

[1] In view of the fact that the percentage of typhoid in either group is very small, absolutely, the distribution of random errors in second samples will hardly be normal, so that probable errors, calculated by the usual formula, need care in interpretation. (*See* Karl Pearson. Phil. Mag., page 365, March, 1907.)

Expressing the ratio of one group-incidence to another gives the result of one case in an inoculated man to 5·6 cases among non-inoculated men, or, to put this mass of figures into still plainer language, they show that typhoid fever was between five and six times as common in the non-inoculated as in the inoculated.

Columns 21 and 22 show the case-mortality per 100 in each unit in the respective groups and also the case-mortality of the whole number of cases which occurred in each group. The latter figures will be seen to be **8·9** per cent. in the inoculated and **16·9** per cent. in the non-inoculated. Too much stress should not, however, be laid on these figures as the total number of cases dealt with is too small and there is so great a disproportion between the number of cases in the two groups, but at the same time attention may again be directed to the report of the Sub-Committee (*see* Chapter IV., Part II.) who concluded from their detailed study of the individual cases that there was evidence that previous inoculation resulted in modifying the severity of a subsequent attack of enteric. It is probable, therefore, that the lower rate of mortality indicated by these figures in the case of the non-inoculated although statistically unsound is practically correct.

Leaving on one side the statistical validity of the figures it will be admitted that it is not easy to explain the facts that in the smaller group of **8,936** non-inoculated men **46** have died from enteric fever, while in the larger group of **10,378** men there have been but **5** deaths from this disease, on any other grounds than the protection afforded by inoculation.

It may be noted, in concluding this chapter, that in units Nos. 11, 16 and 18 no cases of enteric fever occurred in either group during the period under review. The numbers therefore swell the general total without affording any information as to the effects of inoculation. The subtraction of their totals from the whole, however, only influences the final results fractionally since the number of inoculated and non-inoculated men in these three regiments is fairly evenly balanced.

TABLE

The Incidence of Enteric Fever

No.	Unit.	Medical officer.	Last station of unit.	Date of arrival at first foreign station.	Date of latest report.	Total period under observation. Yrs.	Mths.
1.	2.	3.	4.	5.	6.	7.	
1	2nd Bn. Royal Fusiliers	Smallman ..	Secunderabad	Dec., 1904	June, 1908	3	6
2	17th Lancers	Luxmoore..	Meerut ..	Oct., 1905	Aug., 1909	3	10
3	12th Brigade, Royal Horse Artillery.	Lithgow ..	Sialkot ..	Dec., 1905	July, 1908	2	7
4	14th Hussars	Fawcett ..	Bangalore ..	Sept., 1906	July, 1909	2	10
5	3rd Bn. Coldstream Guards	Graham .. Erskine ..	Khartoum ..	Oct., 1906	Aug., 1909	2	10
6	2nd Bn. Leicestershire Regt. ..	Sherren ..	Belgaum ..	Oct., 1906	July, 1909	2	9
7	2nd Bn. Dorsetshire Regt. ..	Anthonisz ..	Madras ..	Nov., 1906	June, 1909	2	7
8	1st Bn. Connaught Rangers ..	O'Carroll ..	Dagshai ..	Mar. 1907	July, 1909	2	4
9	2nd Bn. Bedfordshire Regt.	Drew .. Spencer ..	Gibraltar ..	Sept., 1907	June, 1909	1	9
10	13th Brigade, Royal Horse Artillery.	Littlejohns	Pretoria ..	Oct., 1907	July, 1909	1	9
11	1st Bn. Suffolk Regt.	Mulligan ..	Malta.. ..	Nov., 1907	June, 1909	1	7
12	1st Dragoon Guards	Stevenson..	Amballa ..	Dec., 1907	June, 1909	1	6
13	1st Bn. Lancashire Fusiliers ..	Howell ..	Meerut ..	Dec., 1907	July, 1909	1	7
14	3rd Bn. Worcestershire Regt. ..	Forsyth ..	Wynberg ..	Dec., 1907	Sept., 1908	0	9
15	1st Bn. Yorkshire Regt... ..	O'Grady ..	Alexandria ..	Jan., 1908	July, 1909	1	6
16	3rd Bn. King's Royal Rifles ..	Leslie ..	Crete	Feb., 1908	June, 1909	1	4
17	7th Dragoon Guards	Gibbon ..	Cairo	Sept., 1908	June, 1909	0	9
18	6th Inniskilling Dragoons ..	Langrisbe ..	Mhow ..	Oct., 1908	July, 1909	0	9
19	1st Bn. Border Regt.	Johnson ..	Wellington ..	Oct., 1908	July, 1909	0	9
20	2nd Bn. Somersetshire Light Infantry.	Gibson ..	Malta.. ..	Nov., 1908	June, 1909	0	7
21	4th Bn. Worcestershire Regt. ..	Wood ..	Bareilly ..	Nov., 1908	July, 1909	0	8
22	2nd Bn. Liverpool Regt... ..	Mitchell ..	Sabathu ..	Jan., 1909	July, 1909	0	6
23	2nd Bn. Devonshire Regt. ..	Beaman ..	Crete	Jan., 1909	June, 1909	0	5
24	1st Royal Scots	Amy ..	Ranikhet ..	Feb., 1909	June, 1909	0	4
					Average..	1	8

I.

among the 24 "Test Units."

Total strength of unit			Inoculated.				Uninoculated.				Case incidences per 1,000.		Case mortality per 100.	
			Strength.		Enteric.		Strength.		Enteric.					
When medical officer joined.	On arrival first foreign station.	At date of last report.	On arrival first foreign station.	At date of last report.	Total cases.	Total deaths.	On arrival first foreign station.	At date of last report.	Total cases.	Total deaths.	Inoculated.	Uninoculated.	Inoculated.	Uninoculated.
8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.
358	883	1,013	106	198	12	1	777	815	68	9	60·0	83·0	8·3	13·2
490	576	620	130	460	18	2	446	160	96	18	39·1	600·0	11·1	18·7
177	356	330	46	202	0	0	310	128	7	1	Nil	54·6	Nil	14·2
558	599	634	211	377	4	1	388	257	6	2	10·6	23·3	25·0	33·3
708	708	754	90	540	2	0	618	214	10	1	3·7	46·7	Nil	10·0
356	938	910	149	589	5	1	789	321	24	3	8·4	74·7	20·0	12·5
376	1,058	1,108	62	311	1	0	996	797	12	2	3·2	15·0	Nil	16·6
485	485	1,006	300	611	0	0	185	395	7	2	Nil	17·5	„	28·5
793	887	927	294	309	0	0	593	618	7	1	„	11·3	„	14·2
265	363	354	240	235	2	0	123	119	6	0	8·5	50·4	„	Nil
890	890	926	391	412	0	0	499	514	0	0	Nil	Nil	„	„
527	645	620	220	485	3	0	425	135	2	1	6·1	14·8	„	50·0
830	999	959	716	890	2	0	283	69	0	0	2·2	Nil	„	Nil
614	635	633	206	209	0	0	429	424	3	0	Nil	7·0	„	„
794	843	847	439	460	0	0	404	387	3	1	„	7·7	„	33·3
889	880	928	177	217	0	0	703	711	0	0	„	Nil	„	Nil
575	575	561	171	242	0	0	404	319	1	1	„	3·1	„	100·0
433	605	605	397	547	0	0	208	58	0	0	„	Nil	„	Nil
999	998	990	301	422	0	0	697	568	1	0	„	1·7	„	„
486	869	890	283	285	0	0	586	605	5	1	„	8·2	„	20·0
893	1,004	1,004	601	912	4	0	403	92	11	3	4·3	119·5	„	27·2
461	922	909	471	510	2	0	451	399	0	0	3·9	Nil	„	Nil
481	855	905	253	253	0	0	602	652	1	0	Nil	1·5	„	„
542	910	881	561	702	1	0	349	179	2	0	1·4	11·1	„	„
13,980	18,483	19,314	6,815	10,378	56	5	11,668	8,936	272	46	5·39 ±0·48	30·4 ±1·23	8·9	16·9

CHAPTER III.

The Cases of Typhoid Fever among the Inoculated.

(With Table II.)

As it was anticipated that useful information might be derived from a careful study of the cases of enteric fever which occurred in men who had been inoculated, accurate records have been kept in every unit not only of the fact of inoculation but, in addition, of the number and date of the vaccine employed, the number of inoculations, the period which had elapsed between inoculation and attack and many other points. The influence of inoculation in modifying the severity of the attack has been studied by the examination of the temperature charts and the detailed medical history of each case by the Sub-Committee appointed for the purpose, whose report will be found in the next chapter.

Table II., then, will be found to summarise the information which has, from time to time, been communicated to Sir William Leishman by the medical officers attached to each unit in connection with the following points, relating to each of the 56 cases of enteric fever which occurred in inoculated men :—

1. Name of the patient.
2. Total number of inoculations which he had received.
3. The number and date of preparation of the vaccine or vaccines employed.
4. The age of the vaccine at the time at which it was used.
5. The period which had elapsed between inoculation and attack.
6. The nature of the attack, according to the following classification :—

   Very mild.
   Mild.
   Moderately severe.
   Severe.
   Fatal.

The final column of the Table is left for remarks in connection with individual cases.

The Table does not appear to call for many words of explanation. The cases are numbered consecutively, by units, and each man's name is given, with two exceptions, where the medical officer only furnished his initial.

The number of inoculations given to each man varied from one to four. Although two inoculations, at an interval of 10 days, are always advised it has not been possible in all cases to persuade the men to come forward for their second dose, while in some cases illness or absence has been responsible for this second dose being either abandoned or considerably delayed. In other cases a man has been re-inoculated after the lapse of a year or two, either with one dose or with two. In each instance, column 4 will show the particular vaccine which was employed in each inoculation or re-inoculation.

The older vaccines were distinguished by letters, the more recent ones by figures, and in each instance the date on which the sterility of the vaccine was verified is also recorded. In this connection it is important to bear in mind that all vaccines up to and including letter E were prepared according to the old system, in use at the time of the South African war, and all vaccines lettered F, G, &c., as well as all those distinguished by numbers, are what have been referred to throughout this Report as the "new" vaccine, *i.e.*, a vaccine which had been sterilised at a temperature of 53° C. and had been prepared from a young culture of 24—48 hours' growth.

The next column gives the age of the particular vaccine which was employed at each inoculation ; as will be seen, in many instances, this greatly exceeded the period of 3 months which was subsequently fixed on as being the maximum time during which a vaccine could be counted on to retain its full efficacy.

The next gives the period in years and months which had elapsed between inoculation and attack. In cases where more than one vaccine had been used, either at an interval approximating 10 days or in the case of a re-inoculation after many months, the period between each series of inoculations and the subsequent attack is separately recorded.

The other columns call for no explanation.

The principal object in collecting the various items of information summarized in this Table has been to throw light upon such points as the relative efficiency of one inoculation as compared with two or more, the differences, if any, in the apparent protection afforded by different batches of vaccine and other points whose importance had emerged during the experimental work dealt with in the earlier chapters.

The question of effective protection should be judged by the standards described below, and is of obvious importance in considering the cases of the men who, in spite of inoculation, have contracted enteric fever.

The standards of effective protection which have been adopted, either as the result of experiment or experience, are the following. Justification for them is to be found in the detailed account given in the earlier chapters.

1. Two inoculations, at an interval of 10 days, are considered necessary for the conferring of the maximum degree of immunity; men, therefore, who had only received one dose, cannot be regarded as fully protected. In this connection it should be recalled that the first dose of vaccine contains only 500 million bacteria, while the second contains 1,000 million.

2. The vaccine employed should be the "new" vaccine and not one which had been to some extent damaged by the excessive heat formerly employed in sterilization. In connection with this standard it will be noted that in two instances the vaccine which was used is recorded as "Kasauli," which means that the vaccine used in these cases had been prepared at the Pasteur Institute, Kasauli, and no information as to the actual details of preparation was available.

3. The vaccine should not have been more than 3 months old at the time of its employment.

4. It has also been concluded that the protection given by effective inoculation does not last more than 2 years in the great majority of cases, as evidenced by the decline of the protective substances in the blood and statistical information as to protection.

Taking these four standards as evidence of what may be called "effective protection," the Table permits of a determination of how many of these 56 cases were to be classed as effectively protected at the time at which they contracted the disease. Failure to conform to one or other of the four standards will first be considered separately, and then the total of those who fail in one or more respects will bearrived at.

Class 1. *One inoculation only.*—In **8** cases this will be seen to be the case. These cases are Nos. 13, 14, 15, 16, 26, 40, 42 and 55.

Class 2. *Not inoculated with new vaccine.*—Vaccine prepared in the older manner was employed in **16** cases, including the whole of the cases in the Royal Fusiliers. The cases affected are Nos. 1—12, and also Nos. 37, 38, 40 and 41.

In cases 11 and 12 no information could be obtained as to the actual number of the vaccine used, as the men were inoculated on board transports when coming out with drafts to join the regiment in India, and only the fact of inoculation had been recorded in their documents. From the date at which they arrived, however, it was certain that the new vaccine had not then come into use. Cases 23 and 30 were inoculated with "Kasauli," but are *not* included since this vaccine, having been prepared by Lieut.-Colonel Sir David Semple, may with confidence be accepted as effective.

Class 3. *Vaccine used more than 3 months old.*—No case has been included in this class if any one of the doses which he had received at various times was less than 3 months old at the time of use. **15** cases fall into this class. Nos. 5, 6, 9, 15, 19, 21, 23, 35, 37, 38, 40, 41, 42, 45 and 55.

Class 4. *Attack 2 years or more since inoculation.*—This happened in **3** cases. Nos. 22, 25 and 26.

Examining now the whole of the 56 cases from the point of view of failure to comply with one or more of these four standards of effective protection, it appears that **30** are included in one or more of the classes, and only **26** come under the above definition of having been effectively protected. The details are as follows:—

Included in one class only..	19
Included in two classes	10
Included in three classes	1
Not included in any class	26
Total ..	56

It will thus be seen that although **56** inoculated men contracted enteric fever out of the total of **10,378**, only **26** of these cases were in men who might have been considered as effectively protected by their inoculations.

No exact figures are available for comparison as to the numbers of men effectively protected and not effectively protected in the units, but it may safely be said that the enormous majority were effectively protected in the sense described; for instance, in many units there was no single case of a man who had received only one inoculation and, since the year 1906, no dose of the old vaccine has been given to any soldier. Again, the regulations about the non-employment of any vaccine more than 3 months after its date of preparation have been strictly enforced since the importance of this was recognized, and in the case of the majority of the units it was only by accident or oversight that an odd dose of a vaccine older than this has been given.

The fact that considerably more than half of the 56 cases are shown by this table not to conform to the definition of effective protection is one of great significance and should not be lost sight of in assessing the general protective value of the new vaccine from the results displayed in Table I.

TABLE II.

TABLE

The cases of Enteric Fever

	Regiment and name.	Number of inoculations.	Number and date of vaccine.	Age of vaccine at time of use.	Yrs.	Mths.
	2nd Bn. Royal Fusiliers.					
1	Wilson	2	B.—Oct., 1904		0	1
2	Keats	4	B.—Oct., 1904 ..	1st two	0	1
				2nd two.. ..	1	1
3	Longhurst	3	B.—Oct., 1904 ..	1st two	0	1
				3rd	1	1
4	Smith	4	B.—Oct., 1904 ..	1st two	0	1
				2nd two.. ..	1	1
5	Buckley	2	B.—Oct., 1904		1	0
6	Luddington	2	B.—Oct., 1904		1	0
7	Coleman	3	B.—Oct., 1904 ..	1st two	0	1
				3rd	1	0
8	Arbour	4	B.—Oct., 1904 ..	1st two	0	1
				2nd two.. ..	1	0
9	Hewitt	2	B.—Oct., 1904		1	0
10	Lowe	2	E.—July, 1905	?		
11	Whistler	?	?	?		
12	Bone	?	?	?		
	17th Lancers.					
13	Cotter	1	F.—Aug., 1905		0	1¼
14	Roebuck	1	F.—Aug., 1905		0	1¼
15	Groom	1	28.—July, 1907		0	4½
16	Richards	1	42.—Jan., 1908		0	2
17	Leverton	3	1st two .. G.—Nov., 1905 ..	1st two ..	1	1
			3rd .. 10.—May, 1806 ..	3rd	0	2¼
18	Richmond	2	31.—Aug., 1907		0	1
19	Orchard	2	G.—Nov., 1905		1	1
20	Jebb	2	50.—Aug., 1908		0	1
21	King	2	G.—Nov., 1905		1	1
22	Poole	2	F.—Aug., 1905		0	1¼
23	Thornton	2	Kasauli.—April, 1908		0	6
24	Lawson	2	31.—Aug., 1907		0	1
25	Cooper	4	1st two .. F.—Aug., 1905..	1st two ..	0	1
			2nd two .. G.—Nov., 1905..	2nd two ..	1	1
26	Payne	1	10.—May, 1906		0	2½
27	Holley	2	42.—Jan., 1908		0	2
28	Pulbrook	2	31.—Aug., 1907		0	1
29	Smith	3	1st two .. 12.—June, 1906 ..	?		
			3rd .. 44.—Mar., 1908 ..	3rd	0	2
30	Lancaster	2	1st .. 50.—Aug., 1908 ..	1st	0	1¾
			2nd .. Kasauli.—April,1908	2nd	0	5
	14th Hussars.					
31	Stevens	2	22.—Mar., 1907		0	3
32	Murch	2	10.—May, 1906		0	3
33	Lockwood	2	1st .. 10.—May, 1906 ..	1st	0	3
			2nd .. 11.—May, 1906 ..	2nd	0	3
34	Chester	2	10.—May, 1906		0	3
	3rd Bn. Coldstream Guards.					
35	Prescott	2	11.—May, 1906		0	4¾
36	Williams	3	1st two .. 10.—May, 1906 ..	1st two ..	0	4¼
			3rd .. 26.—May, 1907 ..	3rd	0	1¼

II.

among the Inoculated.

Period between inoculation and attack.	Nature of attack.	Remarks.
Yrs. Mths.		
1 4	Severe	Relapse.
1st two .. 1 4	Mild	Duration of fever, 18 days.
2nd two .. 0 4		
1st two .. 1 5	Very mild	Duration of fever, 12 days.
3rd .. 0 6		
1st two .. 1 9	Moderately severe	Died of cholera during convalescence.
2nd two .. 0 10		
1 0	Mild.	
1 0	Mild.	
1st two .. 1 10	Mild	Duration of fever, 14 days.
3rd .. 1 0		
1st two .. 2 0	Very mild.	
2nd two .. 1 2		
.. 1 0	Very mild.	
1 10	Died	Fatal hæmorrhage.
?	Mild	Complicated with malaria.
?	Severe	Malaria. Thrombus.
0 $2\frac{1}{2}$	Mild.	
9 $6\frac{1}{4}$	Moderately severe	Thrombus.
0 $1\frac{1}{4}$	Mild.	
11 days	Very mild	Malaria.
1st two .. 2 5	Moderately severe	Paratyphoid A?
3rd .. 1 10		
1 2	Moderately severe.	
1 11	Moderately severe	Relapse.
0 $1\frac{1}{2}$	Mild.	
1 11	Severe.	
3 $1\frac{1}{2}$	Died.	
0 1	Moderately severe.	
1 3	Very mild	Duration of fever, 8 days.
1st two .. 3 4	Mild.	
2nd two .. 2 0		
2 7	Mild	Duration of fever, 12 days.
0 11	Died	Following gonorrhœa.
1 6	Mild	Complicated with malaria.
1st two .. 1 5	Very mild	Slight relapse.
3rd .. 0 10		
1st .. 0 8	Very mild	Duration of fever, 8 days.
2nd .. 0 8		
1 4	Very mild.	
0 11	Very mild.	
1st 1 10	Died.	Perforation.
2nd 1 10		
1 0	Very mild	Duration of fever, 5 days.
1 0	Very mild	Duration of fever, 13 days.
1st two .. 2 0	Very mild	Duration of fever, 12 days.
3rd .. 1 1		

TABLE

	Regiment and name.	Number of inoculations.	Number and date of vaccine.	Age of vaccine at time of use.	Yrs.	Mths.
	2nd Bn. Leicestershire Regt.					
37	White	2	E.—Aug., 1905		1	2
38	Walker	2	E.—Aug., 1905		1	7
39	Bryan	2	10.—May, 1906		0	3
40	Private E	1	E.—Aug., 1905		1	3
41	Private D.	2	E.—Aug., 1905		1	7
	2nd Bn. Dorsetshire Regt.					
42	Plenty	1	11.—May, 1906		0	4½
	13th Brigade, Royal Horse Artillery.					
43	O'Toole	2	1st .. 16.—January, 1907 2nd .. 27.—July, 1907	1st .. .. 0 7 2nd 0 2		
44	Pringle	2	1st .. 26.—May, 1907 2nd .. 27.—July, 1907	1st .. .. 0 4 2nd 0 2		
	1st King's Dragoon Guards.					
45	Bennett	2	33.—Sept., 1907		0	3½
46	Christian	2	32.—Sept., 1907		0	0½
47	Fawcett	2	50.—Aug., 1908		0	0½
	1st Bn. Lancashire Fusiliers.					
48	Brennan	2	34.—Sept., 1907		0	2
49	Whiteside	2	1st .. 30.—July, 1907 2nd .. 31.—Aug., 1907	1st .. .. 0 1 2nd 0 1		
	4th Bn. Worcestershire Regt.					
50	Llewellyn	2	50.—Aug., 1908		0	1
51	Bate	2	1st .. 12.—Jan., 1907 2nd .. 16.—Jan., 1907	1st .. .. 0 1½ 2nd 0 1		
52	Bishop	2	51.—Aug., 1908		0	1
53	Ashby	2	48.—June, 1908		0	2
	2nd Bn. Liverpool Regt.					
54	Chesterman	2	65.—Feb., 1909		0	2
55	Hardinge	1	25.—April, 1907		0	7
	1st Bn. Royal Scots.					
56	Lindsay	2	23.—April, 1907		0	3

II.—*continued.*

Period between inoculation and attack.				Nature of attack.	Remarks.
	Yrs.	Mths.			
	0	5½		Very mild.	
	0	6		Mild.	
	1	4		Died	4 months between 1st and 2nd inoculations.
	1	8½		Mild.	
	1	6		Very mild	Duration of fever, 14 days.
	0	7		Very mild	
1st	0	5½		Very mild	6 days' fever, paratyphoid ?
2nd	0	5			
1st	1	7½		Very mild	5 days' fever, paratyphoid ?
2nd	1	7½			
	0	5		Very mild.	
	1	1		Mild	2½ months between 1st and 2nd inoculations.
	0	5		Mild	Duration of fever, 11 days.
	1	4½		Mild	Slight relapse.
1st	1	6		Mild.	
2nd	1	6			
	0	4		Very mild	Duration of fever, 10 days.
1st	1	10		Very mild	Duration of fever, 6 days.
2nd	1	10			
	0	7		Moderately severe.	
	0	9		Mild	Duration of fever, 12 days.
	0	1		Very mild	Complicated by malaria.
	1	6		Mild.	
	1	11		Very mild.	

CHAPTER IV.

Report of the Sub-Committee on Typhoid Cases.

1. The following Sub-Committee was appointed at the 16th meeting of the Anti-Typhoid Committee, on 2nd July, 1908 :—

Dr. James Galloway.
Dr. R. Foord Caiger.
Lieut.-Colonel Sir W. Leishman.

2. They were requested to make an examination of the case-sheets and temperature charts of the 208 cases of enteric fever shown in the return presented to the Committee by Lieut.-Colonel Leishman in August, 1908, and to report to the Committee the results of this examination.

3. The collection of the documents was undertaken by the War Office and they were forwarded to Colonel Leishman as soon as they were received from the various foreign stations at which the test units were or had been stationed.

4. Since the process of collection took a considerable time the total number of cases had, in the meanwhile, increased and the sub-Committee were therefore able to study the particulars of 50 additional cases, making a total of 258.

The documents, then, which have been studied by the Sub-Committee are as follows :—

Non-inoculated cases	202
Inoculated cases	56
Total	258

5. The Sub-Committee have no hesitation in expressing the opinion that these were genuine cases of enteric fever; indeed the records tend to show that, in the large majority of the cases, the attack was typical. Besides the information gained from the temperature charts and the clinical histories recorded in the case sheets they also noted the following points. 1st. Widal's reaction had been carried out in every instance. 2nd. In a large proportion of the cases the attempts which had been made to isolate the typhoid bacillus during life from the blood or excreta had been successful. 3rd. In those fatal cases in which an autopsy was permitted the post-mortem characters recorded were typical of enteric fever.

6. It was felt that a closer study of the details of these 258 cases might disclose information bearing upon the relative severity of the disease in the inoculated and the non-inoculated, respectively ; this was therefore undertaken on the following lines :—

Each case, after a careful consideration of all the available evidence, was referred to one of the following five classes :—

Class 1.—" Very mild cases." In this were included such as showed an exceptionally brief period of pyrexia, not exceeding 14 days, and in which the range of the temperature has been low. They had also been completely free from complications and had shown no severe signs of toxæmia.

Class 2.—" Mild cases." In these the duration of pyrexia was somewhat longer than in Class 1, averaging 18 to 21 days, but resembling Class 1 in the moderate height of the temperature, the freedom from complications and signs of toxæmia.

Class 3.—" Moderately severe cases." Such were cases of average severity and duration, the pyrexia lasting 3 weeks or longer ; the degree of pyrexia was also higher than in the former classes and the patients had shown signs of toxæmia, though not of a severe nature. Cases, also, which, although mild in the initial attack, had been followed by a relapse, as well as such as had been complicated by the occurrence of phlebitis, bronchitis, hæmorrhage, &c., if not exceptionally severe, were also included in this class.

Class 4.—" Severe cases." In this class were placed all cases in which it was clear that the patient's life had been in danger, either from hyper-pyrexia, prolonged pyrexia, severe toxic symptoms, dangerous complications, severe relapses or greatly protracted convalescence.

Class 5.—" Fatal cases." No attempt was made in such cases to distinguish between those in which the attack appeared to be of mild nature, death occurring from some accident such as hæmorrhage, and those in which death was due to the intensity and

severity of the infection. The small number of fatal cases in the inoculated would have rendered any comparisons of this nature valueless.

The Sub-Committee were in agreement in each instance as to the particular class to which each case should be assigned, and, in the majority of the cases, the classification was made independently by two members.

The following is the result of the classification :—

Class.	Inoculated.	Non-inoculated.	Total.
1. "Very mild"	17	26	43
2. "Mild"	20	33	53
3. "Moderately severe"	11	60	71
4. "Severe"	3	49	52
5. "Fatal"	5	34	39
Totals	56	202	258

It will be seen from this table that the average severity of the attack has been greater in those who had not been inoculated than in those who had been inoculated and subsequently contracted enteric. This is still more apparent when the relative percentages of the classes are considered and contrasted, as has been done in the following table. In this the milder cases comprised in Classes 1 and 2 have been grouped together and contrasted with a similar grouping of the moderately severe, severe and fatal cases included in Classes 3, 4 and 5.

	Percentage of combined "very mild" and "mild" cases.	Percentage of combined "moderately severe," "severe" and "fatal" cases.
Inoculated	66·1 per cent.	33·9 per cent.
Non-inoculated	29·3 "	70·7 "

General conclusions.

1. The examination of the case-sheets and temperature charts of the 258 cases proves that they were genuine cases of enteric fever.

2. The average severity of the inoculated cases was considerably less than that of the non-inoculated.

JAMES GALLOWAY.
F. FOORD CAIGER.
W. B. LEISHMAN.

December, 1909.

PART III.

FINAL SUMMARY AND CONCLUSIONS.

The results of the more important of the various investigations undertaken for the Committee, and the conclusions drawn from them, may be briefly summarised as follows:—

A.—Experimental Results.

1. The development of immunity against the typhoid bacillus, as indicated by quantitative investigation of the various anti-bodies produced, was exhaustively studied both in man and animals.

2. The relative efficiency of vaccines prepared in different ways was then measured. Vaccines made by a variety of methods were compared, but none found to possess material advantage over that of Sir A. E. Wright, provided the temperature employed for sterilization be reduced to 53° C.

3. A large number of methods which had been suggested for the standardization of vaccines were critically examined, and the enumeration of the number of bacteria in a given volume of vaccine was adopted as the most accurate and convenient.

4. The keeping qualities of the vaccine at various temperatures and under practical conditions, such as a voyage through the tropics, have been examined. Vaccines three months old were found to be efficient, but serious deterioration in immunizing value was apparent in those which had been kept six months or upwards.

5. Careful experiments were made upon groups of volunteers, in order to determine the most effective system of dosage, viz., one affording adequate protection with the minimum of temporary disability to the soldier.

B.—Statistical Results.

The histories, as regards typhoid fever, of 19,314 soldiers, whose average period of service abroad was 20 months, were carefully followed, and every precaution possible was taken to verify the diagnosis bacteriologically. Of this number, 10,378 were inoculated, and 8,936 not inoculated. The case incidence of typhoid fever among the inoculated was 5·39 per mille, and among the non-inoculated 30·4 per mille.

There is no reason for supposing that this difference can be attributed to a want of homogeneity between the two groups. The age distribution among inoculated and non-inoculated was approximately the same. They were intermingled and lived under identical conditions.

The experience of Lieut.-Colonel Sir W. B. Leishman and the inoculating officers lends no support to the view that soldiers who, from their character and habits, might presumably be more likely to incur the risk of infection, present themselves for inoculation in smaller numbers than their more careful comrades.

In the opinion of the Committee the substantial difference in the incidence can only be attributed to inoculation.

RECOMMENDATIONS.

1. Every measure which may be considered practicable, should be employed to extend the practice of anti-typhoid inoculation in the Army. In the opinion of the Committee its universal application is desirable.

2. Research work on preventive inoculation against typhoid fever, a subject of special importance to the Army, should be continued.

CHARLES J. MARTIN, *Chairman.*
JOHN ROSE BRADFORD.
F. FOORD CAIGER.
GEORGE DEAN.
JAMES GALLOWAY.
WILLIAM B. LEISHMAN.
ROBERT BRUCE LOW.

EDMOND T. GANN, *Secretary.*
28*th October*, 1912.

[Colonel Sir David Bruce, Knt., C.B., F.R.S., M.B., was abroad when this Report was signed.]