Report on the blood changes following typhoid inoculation / by W. B. Leishman.

ANTI-TYPHOID INOCULATION COMMITTEE.

REPORT ON THE BLOOD CHANGES FOLLOWING TYPHOID INOCULATION,

BY

MAJOR W. B. LEISHMAN, R.A.M.C.,
Professor of Pathology, Royal Army Medical College.

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PREFACE.

In submitting this Report to the Anti-typhoid Committee, I would wish, in the first place, to emphasise the nature of the work performed by my colleagues, Captain W. S. Harrison, Lieut. A. B. Smallman, and Lieut. F. M. G. Tulloch, R.A.M.C. Almost the whole of the work carried out at Aldershot was done by these officers, as in this I was able personally to assist them to but a small extent. The exacting nature of this work will, I think, be evident from the report, and I am anxious that the Committee should not under-estimate the extent to which I am indebted to these officers for the skill and unremitting labour with which they carried out the investigation.

To Captain Harrison I am further indebted for his help in drawing up many of the Protocols and Charts.

PART I.

At the end of September, 1904, as soon as sanction had been obtained to the proposed investigation, the Commanding Officer of the 2nd Royal Fusiliers was communicated with, and I was informed that the majority of the men who were to proceed to India on November 23rd were on furlough, and would not return to Aldershot until October 18th and 19th. It was accordingly arranged that I should give a lecture to the men on October 20th, and that the inoculations should be carried out upon the volunteers on the following day.

The time thus left at our disposal was employed at the Pathological Laboratory of the Royal Army Medical College :—

1st.—In the selection and preparation of the Vaccine.

2nd.—In the selection and practice of the various technical processes required in the investigation.

3rd.—In the attempted elucidation of some of the problems that arose in connection with these methods.

4th.—In control experiments with normal blood.

We further inoculated ourselves with a sample Vaccine to assist us in the fixing of the dosage to be employed on the regiment. Details of some of this preliminary work are incorporated in the report, in so far as they are germane to the general investigation.

On October 16th, Captain Harrison, Lieut. Smallman, and Lieut. Tulloch proceeded to Aldershot accompanied by a N.C.O. and man of the R.A.M.C. from the staff of the Pathological Laboratory of the Royal Army Medical College, taking with them the equipment which I had found to be needed to supplement that already in the District Laboratory at the Cambridge Hospital, Aldershot. This laboratory was placed at our disposal by the kindness of Surg.-General McNamara, P.M.O., Aldershot, and Major Elkington, District Sanitary Officer.

Work was at once started here to put into practice the daily routine of the experimental work we had laid down for ourselves, using as material the "pooled" blood of normal men, so that, once the actual research had commenced, there should be as few hitches due to defective organisation as possible, at least in so far as the difficulties likely to be met with could be foreseen.

The investigation commenced on October 21st with the first inoculations of the volunteers and it was continued daily till November 17th, after which date the men could no longer be spared from their regimental duties, pending embarkation.

I am glad to take this opportunity of expressing my thanks to the commanding officer and officers of the 2nd Royal Fusiliers for the readiness with which they met our requirements and for their sympathetic assistance, without which our task would have been rendered infinitely harder, if not altogether impossible.

Selection and Preparation of the Vaccine.

"*A*." *The strain of Bacillus Typhosus selected.*

It was originally intended to employ for this purpose a strain "G," isolated from the spleen at Netley 5 years ago, which had been largely employed by Dr. Wright and myself in the preparation of vaccine, and is still employed by Dr. Wright for this purpose. In our preliminary work, however, it was found to possess the disadvantage of being a strain which could only be emulsified from an agar culture with great difficulty and at the sacrifice of more time than we were likely to be able to afford. Further experiment resulted in the selection of another strain, "R," of similar origin and of about the same age, which had also been extensively employed at Netley in the preparation of vaccine. This strain was one which furnished a very even and satisfactory emulsion from an agar culture, and was thus more suitable for some parts of the delicate experimental work which lay before us. Both these strains being of low virulence, and of proved suitability for inoculation, preference was accordingly given to that which promised to give more regular results in our test experiments, and the strain "R" was therefore employed both in the preparation of the vaccine and in the daily quantitative tests of the protective substances developed in the blood of the inoculated.

"*B.*" *The Vaccine.*

This was prepared on lines similar to those employed by Dr. Wright and myself at Netley, with the exception that a young broth culture was employed in place of one 10–14 days old, as was our custom then. This modification was adopted after a consultation with Dr. Wright, who was good enough to give me full particulars of his present methods of preparing vaccine, and whose advice and experience were placed at my disposal on this and several other points with his usual readiness.

I need not give details of the preparation of the vaccine except where these happen to differ from the method described in the *British Medical Journal*, January 20th, 1900, by Dr. Wright and myself. The culture flasks were incubated for 42 hours at 37° C. After 24 hours, the growth was found to be too weak and would have necessitated the inoculation of larger quantities of vaccine than are convenient; the culture was therefore replaced in the incubator for 18 hours. After samples had been drawn for the purposes of enumeration of the bacilli and of re-testing the purity of the growth, the contents of the flasks were mixed in a mixing jar and sterilised in a water bath at an average temperature of 62° C. maintained for 15 minutes. The temperature was controlled by the use of a second mixing jar filled with water and fitted up in the same way as that containing the vaccine, but with a thermometer passed through the bung into the centre of the fluid. This jar was previously kept for some hours beside the vaccine jar, so that the temperature of each might be identical at the time they were placed in the water bath. A careful check was kept upon the temperature in the control jar, which, during the 15 minutes, did not rise above 63·5° C. nor fall below 60° C.

When cool, samples were drawn for testing and, after proof of sterility by aërobic and anaërobic cultures, ·5% of Lysol was added.

The vaccine was bottled in the manner described in the above-mentioned article.

"*C.*" *Standardization of the Vaccine.*

Dr. Wright informed me that, using a 24-hour broth culture of a known and proved strain of *Bacillus typhosus*, he now depends for standardization upon an enumeration of the germs by the blood-counting method described by him in the *Lancet* of July 5th, 1902, which, in his hands, gives regular and reliable results.

This method we accordingly put into practice with various trial samples of broth and agar vaccines, but although at times we obtained uniform results which were controlled by a living count—made by dilution and plating out on agar—we were unable to obtain consistent and satisfactory results, especially in the case of broth vaccines, where errors of from 50 to 100% in counts of the same film made by different observers were by no means uncommon. All the devices

recommended by Dr. Wright, and many others, were employed towards securing a perfect blood film which in all its parts should represent accurately the relation of the number of germs to the number of red blood corpuscles, but without giving us any greater confidence in the results obtained. The chief factors which appear to interfere with the accuracy of the method are:—

1st. The difficulty in securing a perfect film in which the ratio of germs to cells shall be constant throughout.

2nd. The clumping or agglutination which frequently occurs, especially in broth cultures, leading to great irregularities in the enumeration of a series of microscopic fields.

3rd. The part played by the bacteriolytic action of the blood fluids which, at times, undoubtedly leads to an under-estimation of the number of germs.

Still, at times, the results obtained were apparently trustworthy, especially with agar emulsions of the strain "R," and in several instances such counts were confirmed by the independent observations of three of us, and checked by the alternative method of living enumeration which will be detailed below. Some of these agar emulsions, of whose strength we were thus able to be fairly confident, served a useful purpose in the final estimation of the strength of the vaccine employed at Aldershot and will be referred to below.

Counting of a culture by dilution and plating on agar depends for its accuracy on the freedom of the broth culture or emulsion from clumps of bacilli and, further, upon the assumption that the number of dead germs in a young culture of 24 to 48 hours is small enough to be neglected. This method was employed daily in the standardization of the test-tube cultures used in the analytical work at Aldershot and yielded satisfactory results, but in the measurement of the actual vaccine, grown in flasks on a large scale and mixed, the figures obtained were not to be relied upon as the microscope showed a considerable quantity of small clumps in the culture, each of which, of course, when inoculated on agar, would develop into a single colony and the result be read as 1 germ.

We obtained some assistance in controlling the figures arrived at by the enumeration of the germs in the vaccine from our Chairman, Dr. C. J. Martin, at whose suggestion estimations were made of the weight of the dried bacterial bodies in a measured quantity of vaccine and a correlation obtained for this weight and the number of bacteria as estimated by the living and dead counting methods. These estimations Dr. Martin was good enough to carry out for me on a number of trial emulsions and vaccines, and the consistency of the results obtained appears to promise that, when such a correlation has once been satisfactorily obtained, this method may be of great help in estimating the strength of a vaccine.

Dr. Martin arrived at his correlation from the results he obtained with two very strong agar emulsions of *B. typhosus* in normal saline

solution, these emulsions having been made and counted by us, in one instance by both the living and blood methods, in the other by the blood method only, with fairly uniform results. The figures are as follows:—

1.—*Agar Emulsion "A" of September 30th.*

Counts, made at Royal Army Medical College:—

A. Living Method (by dilution) .. .. 32,000 millions per c.c.

B. Blood Method (by red B.C.):—

I. by A.B.S., 26,240 millions } Av. 30,390 ,, ,,
II. ,, W.B.L., 34,540 ,, }

Weight Correlation, made at Lister Institute by Dr. Martin:—

I. Residue from 5 c.c. .. ·0176 gm. } Dry residue per c.c.
II. ,, ,, .. ·0175 ,, } ·0035 gm.

2.—*Agar Emulsion "B" of October 4th.*

Counts, Living method. Made at R.A.M. College:—

1 by A.B.S. .. 13,040 millions per c.c } Av. 9,596 millions per c.c.
2 ,, W.S.H. .. 10,000 ,, ,, }
3 ,, F.T. .. 5,750 ,, ,, }

Weight Correlation:—

Dry residue per c.c. .. .. ·00113 gm.

Translating this figure into germs from the correlation obtained with agar emulsion "A" gives 10,300 millions per c.c., or within 8% of the value obtained by taking the average of three independent counts by different workers using Dr. Wright's blood method—viz.: 9,596 millions per c.c.

Working on the assumption that the correlation obtained from these agar emulsions was accurate, Dr. Martin further dried and weighed for me three samples of broth vaccines which I will call "A," "B" and "C," made and sterilised under identical conditions, with the following results:—

"A."—*Unlysolized.*

Estimation I. Residue from 15 c.c. .. ·0031 gm. } Av. ·0033 gm.
,, II. ,, ,, ,, .. ·0034 ,, }
,, III. ,, ,, ,, .. ·0036 ,, }

"B."—*Lysolized* (·5% Lysol).

Estimation I. Residue from 15 c.c. .. ·0080 gm. } Av. ·0081 gm.
,, II. ,, ,, ,, .. ·0082 ,, }
,, III. ,, ,, ,, .. ·0083 ,, }

"C."—1. *Unlysolized Sample.*

Estimation I. Residue from 15 c.c. .. ·0031 gm.
„ II. „ „ „ .. ·0033 „ } Av. ·0032 gm.

2. *Lysolized Sample* (·5% Lysol).

Estimation. Residue from 15 c.c. .. .. .. Av. ·0078 gm.

The difference which the addition of lysol would make was not anticipated when the estimation of "B" was undertaken, so a control experiment was done with two samples of a third vaccine, "C," the one unlysolized, the other after the addition of ·5% lysol. It will be seen from the above figures that the weight of dried bacterial bodies in 15 c.c. of "A" and "C" was practically identical and, further, from a comparison of the weight of the lysolized samples of "B' and "C" it may fairly be inferred that the weight of bacterial bodies in 15 c.c. of an unlysolized sample of "B" would have been about ·0033 gm.

The weight, then, of dried bacterial bodies in 15 c.c. of each of these three vaccines was as follows:—

"A"	.. ·0033 gm.	..	observed.
"B"	.. ·0033 „	..	estimated.
"C"	.. ·0032 „	..	observed.

Putting the correlation figure obtained from the strong agar emulsions into operation, this weight would represent approximately 1,700 million bacilli in 1 c.c. of each of these vaccines.

I have quoted these experiments at some length from the important bearing they have upon the standardization of the vaccine employed at Aldershot, which was the "B" Vaccine of the above series. Owing to the mistake of a subordinate, when sterilizing the rubber caps used for bottling, vaccine "A" was contaminated, subsequent to its sterilization and standardization, therefore "B" was prepared in precisely the same manner, and, subsequently, "C," in order to further control the standardization of "A" and "B" and to determine the effect which the addition of lysol had upon the weight of the bacterial sediment.

Careful experiments were made with all three vaccines to determine the numbers of germs by dilution (living count) and by Dr. Wright's blood method, but it was felt that the results obtained were unreliable owing to the presence of numerous clumps of bacilli. The average result of al counts of "A" was 750 millions per c.c., and of "B" 650 millions per c.c., while counts of "C" were quite unreliable.

To arrive at a practical conclusion of the real strength of "A" vaccine we put it to the test by inoculating ourselves with different doses, 3 receiving 1 c.c. and the fourth ·1 c.c.

The local and general reactions in the case of the 1 c.c. dose were severe and prolonged, lasting for 4-5 days, while with the ·1 c.c. dose

the local reaction was marked and the general reaction moderate, most symptoms disappearing in 48 hours. From these results and from my experience of the effects of inoculation, as well as from the symptoms described by Dr. Wright as following upon a first inoculation of 750-1,000 million bacteria, I have little doubt that the figures we obtained by counting methods for "A" vaccine considerably underestimated its strength, and that the estimate, founded on Dr. Martin's correlation, of 1,700 million per c.c. afforded a more accurate measure of the number of germs contained in this vaccine "A."

The strength of vaccine "B," which was actually employed at Aldershot, was therefore taken as being identical with that of "A," and the dosage was fixed on the assumption that it contained 1,700 million dead typhoid bacilli in 1 c.c.

It may be noted here that, judging from the effects of the inoculations with "B" vaccine, and the development of protective substances in the blood, the above estimate of its strength appeared to be well founded.

"*D.*" *Dosage.*

In accordance with the scheme of dosage approved by the Committee the following quantities of Vaccine "B" were used in the inoculations:—

1. For a small group of volunteers to be inoculated with a comparatively large dose:—

"A" Group.

1st Inoculation ·66 c.c. = 1,133 million bacteria.
2nd „ 1·25 c.c. = 2,125 „ „

2. For the general body of volunteers:—

"B" Group.

1st Inoculation ·33 c.c. = 566 million bacteria.
2nd „ ·66 c.c. = 1,133 „ „

3. For a small group of volunteers to be inoculated with a small dose:—

"C" Group.

1st Inoculation ·1 c.c. = 170 million bacteria.
2nd „ ·2 c.c. = 340 „ „

4. In addition to the above, a certain number of men who had been inoculated 5 years previously were persuaded to come forward for re-inoculation, and these were given a very small dose of vaccine to test the supposed power in such cases of an increased response in the elaboration of protective substances. In this group the dosage employed was:—

"D" Group.

1st Inoculation ·01 c.c. = 17 million bacteria.
2nd „ ·1 c.c. = 170 „ „

"*E*" *Number of men inoculated.*

The total strength of the regiment on sailing for India was 5 officers, 358 W.O.'s, N.C.O.'s and men; but on the date of my lecture —October 20th—only about 200 of these were present.

In all, 106 men volunteered for inoculation, and of these 86 subsequently presented themselves for re-inoculation. This response may, I think, be considered fairly satisfactory under the circumstances, as a certain amount of prejudice against inoculation was found to exist in the battalion owing to the deaths from enteric in South Africa, including those of an officer and several prominent N.C.O.'s who had been inoculated before sailing for the Cape.

"*F*." *Selection of Groups from among the inoculated for the carrying out of the daily blood tests.*

To secure the regular attendance of these groups the co-operation of the regimental authorities was invoked, and sufficient men were induced to volunteer for this purpose from among those inoculated on being excused from morning parade. This plan was found to work satisfactorily, and the men presented themselves daily at the Cambridge Hospital at 7.30 a.m. None had previously suffered from enteric.

"*G*." *Details of the Groups.*

"*A*" *Group.*—6 men, inoculated with ·66 c.c. and re-inoculated with 1·25 c.c. Average age, $20\frac{4}{12}$ years. Average service, $1\frac{8}{12}$ years. This group attended regularly until the date of re-inoculation, when 1 man was dropped out as he was unable to present himself for re-inoculation at the same time as the others.

"*B*" *Group*—8 men, inoculated with ·33 c.c., and re-inoculated with ·66 c.c. Average age, $19\frac{10}{12}$ years. Average service, $1\frac{8}{12}$ years. This group attended regularly and never consisted of less than 7 men.

"*C*" *Group.*—6 boys, inoculated with ·1 c.c., and re-inoculated with ·2 c.c. Average age, $17\frac{1}{12}$ years. Average service, $1\frac{10}{12}$ years. Attended regularly, the smallest number ever present was 4, and it was only on one or two occasions that it fell below 6.

"*D*" *Group.*—5 N.C.O.'s, previously inoculated 5 years ago. Inoculated with ·01 c.c., and re-inoculated with ·1 c.c. Owing to their being often required for duty this group was frequently below full strength, but was never less than 3, except on two occasions when observations were omitted. One N.C.O. dropped out of the group after re-inoculation, having gone on furlough.

The observations upon this group were concluded a few days prior to the others, as these N.C.O.'s could no longer be spared from the multifarious duties in connection with the departure of the regiment.

"*H.*" *The general and local symptoms following the inoculations :—*

1st Inoculations. October 21st.

"*A*" *Group.*—(·6 c.c.) General reaction—moderate, in no case severe; local reaction—pain and soreness complained of at site of inoculation which was marked by redness and swelling.

"*B*" *Group.*—(·3 c.c.) Symptoms as a whole rather less severe than in "A" group. Local reaction much more marked than the general.

"*C*" *Group*—(·1 c.c.) Symptoms moderate, except in the case of 1 boy, aged 15, who was sick and faint in the evening and still unwell next morning, with a temperature of 99·8° F.

"*D*" *Group.*—(·01 c.c.) No appreciable reaction.

The symptoms in all cases disappeared by the end of the second day.

2nd Inoculations. November 1st.

"*A*" *Group.*—(1·2 c.c.) Symptoms, both general and local, as a whole less severe than in "B" and "C" groups. In two cases the men felt absolutely well but for a slight stiffness in the side.

"*B*" *Group.*—(·6 c.c.) In all cases the local reaction was more severe than after the first inoculation, but the general reaction, with one or two exceptions, was milder.

"*C*" *Group.*—(·2 c.c.) Both local and general reactions were more marked than after the first inoculations, and the symptoms were more severe than those following the inoculation of "A" and "B" groups. The boy noted above again suffered most.

"*D*" *Group.*—(·1 c.c.) Slight local reaction only.

In many cases profuse perspiration was complained of on the second night after re-inoculation.

PART II.

The Investigation of the Blood Changes following Inoculation.

In describing the technique which was employed in the various operations it has been thought advisable to do so with considerable detail for the following reasons :—

1st. In order that the value of the results recorded in the protocols and charts may be more accurately assessed.

2nd. That the experiments may be better contrasted with previous work on the same lines.

3rd. That they may be of more service for comparison with any further work that may be done in the direction of improving or modifying the vaccine.

The tests were carried out upon the "pooled" serum of each of the four groups "A," "B," "C," and "D," commencing the day after the first inoculation.

1.—*Method of collecting the blood and of pooling the serum of the Groups.*

The finger was first well scrubbed with a pledget of wool soaked in 2°/₀ lysol, the lysol being removed by the free application of absolute alcohol. When the latter had evaporated, a prick was made with a sterile needle, and the blood collected in a sterile capsule. Four small racks were provided, one for the capsules of each group, and as each capsule was filled it was placed in its appropriate rack. The capsules were then placed in the incubator at 37° C. for two hours, when the serum was separated from the clot of centrifugalization. The serum of each group was then "pooled" by drawing equal volumes from each capsule into the bulb of a sterile pipette which was then sealed off at both ends and vigorously shaken to secure a thorough mixture. Each of the four bulbs was then carefully marked in a permanent manner so as to obviate any chance of subsequent confusion.

In no case was an experiment lost through contamination of the serum collected and pooled in this way.

2.—*Preparation of the daily Stock Culture.*

The stock culture of the "R" strain of *B. typhosus* which was used in the daily estimations was planted out every day at the same hour from the 24 hours' growth in broth inoculated the day before. The same loop was used throughout and no more than the ring of the loop was dipped in the culture, which had previously been well shaken, and used to inoculate the new tube of broth which contained exactly 10 c.c. It was hoped in this way to secure a fairly constant strength of culture for each day's use.

In the earlier part of the investigation it did keep fairly constant, but towards the end the growth became rather more vigorous from the continual daily planting.

3.—*Method of diluting and counting of the Stock Culture.*

This was done daily, as it was felt to be of great importance in connection with the measurement of the bactericidal power of the serum. The method adopted, after much preliminary experimental work, was as follows:—Two series of test tubes were accurately graduated to contain respectively 9 c.c. and 10 c.c., marks being made on the glass corresponding to these volumes of fluid. These were sterilised and kept ready for use. Two pipettes were employed, one calibrated in the manner devised by Dr. Wright to deliver 10 c.mm. of fluid, the other an ordinary 1 c.c. pipette.

In making the 1 in 10,000 dilution of the daily culture, employed in the bactericidal estimations, the culture was first vigorously shaken, 1 c.c. was then added by means of a sterile pipette to a test tube of broth filled up to the 9 c.c. mark, the resulting 1 in 10 dilution was

then in its turn thoroughly mixed and 10 c.mm. transferred by means of the capillary pipette to a test tube containing 10 c.c. of broth, the pipette being washed out with this broth about 15 times and the whole thoroughly mixed.

This method was adopted in preference to the diluting pipette of Dr. Wright as in our hands it gave more accurate results, and also effected some saving of time.

The 1 in 100,000 dilution, used for counting the cultures, was prepared in a similar way by mixing 1 c.c. of the 1 in 10,000 dilution with 9 c.c. of broth.

The counting was performed by inoculating three Agar plates, each with 5 c.mm. of the 1 in 100,000 dilution, the fluid being distributed, drop by drop, over as large a surface of the plate as possible. As the plates were well dried before use, there was no trouble from diffuse growths. The plates were then incubated at 37° C. for 24 hours, and the average number of colonies developed from each of the 5 c.mm. of the 1 in 100,000 dilution was found and multiplied by 20,000,000, the result being taken to represent approximately the number of living bacteria in 1 c.c. of the original culture.

Technique Employed in the Various Quantitative Estimations of the Protective Substances in the Sera.*

1. *The estimation of the agglutinating power of the sera.*

A fresh agar culture of "R" Typhoid, of exactly 24 hours' growth, was emulsified every morning in normal saline solution and approximate uniformity of strength obtained by testing the opacity by means of a card with small, clearly-printed type. Various dilutions of the sera were made with normal saline solution, and equal volumes of these dilutions and of the standardized living emulsions were mixed together, drawn up into a capillary pipette and sealed. The pipettes were set aside at room temperature, and the results were read the following morning. Control tubes of diluted emulsion were put up with each series of serum dilutions.

Marked macroscopic clumping, visible to the naked eye, was taken as evidence of a reaction, and uniformity was secured by this duty being always undertaken by the same observer.

2. *The estimation of the bactericidal power of the sera.*

The general principle of the test adopted was the preparation of a series of dilutions of the serum to be tested and the digestion for one hour at 37° C., of one volume of each of these dilutions with a similar volume of a 1 in 10,000 dilution of the stock broth culture,

* The majority of the technical processes employed were, with occasional modifications, those devised by Dr. Wright, and have been described by him in numerous contributions to the medical journals. A list of references to these articles will be found in his book on "Anti-typhoid Inoculation."—Constable, 1904.

evidence of the effects of bactericidal action being obtained by subsequently inoculating agar plates with the contents of each tube.

Dilutions of each of the pooled sera were made in covered sterile watch glasses, the diluting fluid employed being sterile normal salt solution. The usual dilutions employed were 1 in 5, 1 in 10, 1 in 15, 1 in 20, 1 in 25, 1 in 30, 1 in 35, and 1 in 40, higher dilutions being put up when considered necessary. Equal volumes of each of these dilutions of serum and of the 1 in 10,000 dilution of the broth culture were taken in sterile capillary pipettes, plugged with cotton wool and mixed—thus giving final dilutions of serum of 1 in 10, 1 in 20, 1 in 30, &c.; the sterile cover of the watch-glass served as a convenient mixing surface. The mixture was then drawn up into the pipette in an unbroken column and sealed off, care being taken as far as possible to avoid soiling the inside of the pipette above the upper level of the column of fluid.

The pipettes were then incubated at 37° C. for one hour, at the end of which time the contents were blown on to the surface of agar plates. In opening the pipettes for this purpose the sealed end was first broken off and the tube tilted to allow the fluid to run towards the upper end of the pipette, a further portion of the free end of the tube was then cut off at a point higher than that which marked the original lower level of the fluid. In this way, when the contents were finally blown out on to the agar, they did not pass over a soiled portion of the pipette where, presumably, the serum had not had the same opportunities of acting upon the bacteria as in the mass of the mixture. The plates were incubated at 37° C. for 24 hours and the results as to sterility, or otherwise, were noted. A control experiment was made each day, using equal volumes of normal saline and diluted culture, in order to get an approximate idea of the number of bacteria with which each dilution of the serum had had to deal.

The experiments were kept as far as possible uniform by using approximately the same volume of diluted serum and diluted culture in each series, and these bactericidal tests were carried out simultaneously by two workers to lessen the chance of differences in the strength of the diluted culture, due to further multiplication of the bacteria.

3. *The estimation of the bacteriolytic power of the sera.*

The following strengths of sera were employed—the dilution being made with normal saline solutions:—

Undiluted serum, $\frac{4}{5}$, $\frac{3}{5}$, $\frac{2}{5}$, $\frac{1}{5}$, $\frac{1}{10}$, and $\frac{1}{20}$.

Equal volumes of these dilutions and of a 24 hours' broth culture were mixed, drawn up into capillary pipettes, and incubated for one hour at 37° C. The mixtures were then blown out on to slides, dried, fixed with a saturated solution of perchloride of mercury and stained with methylene blue. The specimens were then examined

microscopically and the results noted, the following classification being adopted:—

No difference from control .. .. .. (0)
Some bacteria unaltered, others spherulated (—)
All bacteria spherulated .. .. .. (+)
Complete disappearance of all bacteria and spherulated forms .. .. (+)

Whatever classification be adopted in measuring bacteriolysis, great difficulty is bound to be experienced, inasmuch as very varying degrees of bacteriolysis must necessarily be placed under the same head. Further sub-division of the above classes would have been possible had time permitted a more lengthy study of each film, but under the circumstances this was felt to be impracticable and it was not attempted.

4. *The estimation of the opsonic power of the sera*

In attempting to determine these substances the technique adopted was founded upon Wright and Douglas' (*Proc. Royal Society*, Vol. 72, 1903, and Vol. 73, 1904) modification of my original method of quantitatively estimating the phagocytic power of the blood. (*British Medical Journal*, January 11th, 1902.) Wright and Douglas showed that in phagocytosis of germs susceptible to the opsonic action of the blood fluids the source of the leucocytes used in the experiment was a matter of subordinate importance, as the phagocytic power of the leucocytes depended, not on any properties inherent to them, but upon the manner in which the germs had been acted upon by the blood fluids.

It should therefore have been possible to measure the opsonic power of the serum of any of the groups by mixing this serum with any freshly washed leucocytes, the result being the same whether the leucocytes used were derived from the blood which furnished the serum or from the blood of any normal individual. Wright and Douglas seem to have demonstrated this fact incontestably in the case of *Staphylococcus pyogenes* and other germs, and as they speak of *B. typhosus* as being eminently susceptible to this opsonic action of the blood fluids, it was hoped that the method described below would have been successful in eliciting any changes which might occur in the opsonic power of the blood during the process of immunisation.

The corpuscles used in the experiments were accordingly taken every morning from one of two normal men, the blood being collected in a capsule and sodium citrate added in the proportion of ·5 %. After centrifugalization the plasma was pipetted off and the corpuscles were thoroughly washed in three changes of normal saline solution.

These washed corpuscles were subsequently used for the testing of the phagocytic powers of the various pooled sera. Two capillary tubes were put up from each group, the first containing the unheated

"active" serum of the group, the second, the same serum "inactivated" by heating to 60° C for 15 minutes to destroy whatever opsonins it might contain.

The following proportions were adhered to throughout:—

Tube 1.—Washed corpuscles	.. .. ..	3 vols.
"Active" serum of group	.. ..	3 ,,
Living emulsion of *B. typhosus*	.. ..	1 vol.
Tube 2.—Washed corpuscles	.. .. ..	3 vols.
"Inactivated" (heated) serum of group		3 ,,
Living emulsion of *B. typhosus*	.. ..	1 vol.

After thorough mixture these tubes were incubated at 37° C. for 15 minutes, films were then made from them and they were stained and counted in the usual way.

The "Phagocytic Index," *i.e.*, the average number of bacteria ingested by the polynuclears, was then estimated, and the ratio between the Phagocytic Indices of tubes 1 and 2 was taken as a measure of the opsonic power of the pooled serum of the group.

It may be added that in the majority of these enumerations the nature of the experiment was unknown to the observer, the slides being merely marked with numbers. This system was adopted to eliminate the sub-conscious mental bias which it is so hard to avoid in this kind of work. Further, in cases in which fewer than 20 polynuclears were counted the result was not recorded.

5. *The Estimation of the "Stimulins."*

Substances which appear to stimulate phagocytosis but differ from opsonins in being thermostable. A short account of the experiments which led me to include in the present investigation a search for evidence of the development of these stimulins will, I hope, shortly be published.* In these experiments the addition of a small quantity of an immune serum to a normal blood was found to stimulate the phagocytic power of the normal polynuclears towards the particular germ which had been used in immunisation. It was further found that the action of these substances, assuming for them a separate existence, was unaffected by heating to 60° for 15 minutes. When Wright and Douglas subsequently published their work on opsonins it was evident from the thermolabile nature of these opsonins that, whatever these stimulating substances might be, they were not identical with opsonins.

The method employed to demonstrate their presence was as follows:—

1. A "Control" tube was put up, containing—
   Normal Washed Corpuscles, 3 vols.
   Normal Heated Serum (60° for 15 minutes), 3 vols.
   Emulsion of Typhoid (living), 1 vol.

* Proceedings of the Pathological Society of London.—*The Lancet*, March 25th, 1905.

2. The serum of each group was tested against this control in tubes containing the following mixture—

Normal Washed Corpuscles, 3 vols.
Normal Heated Serum (60° for 15 minutes), 2 vols.
Pooled Heated Serum of Group (60° for 15 minutes), 1 vol.
Emulsion of Typhoid (living), 1 vol.

In this way all traces of active opsonin were removed unless some might have adhered to the cells in spite of washing or, if the opsonins be of leucocytic origin, have been freshly secreted. The main difference between the two tubes lay in the replacement of one of the volumes of heated normal serum in the control by a corresponding volume of the heated serum of the group. As the experiment progressed this procedure was somewhat modified, as will be noted in describing the results obtained.

The system of recording the results was much the same as that adopted in the opsonic investigations, and will be detailed later.

PART III.

Records of the Determination of the Protective Substances developed in the Sera of the Inoculated Groups.

As these records are fully detailed in the accompanying protocols and charts it will be unnecessary to add much in the way of commentary; this will therefore be mainly confined to an indication of what appear to be the chief points of interest and to a description of a few of the experiments which were undertaken with a view to the elucidation of some points incidental to the investigation.

Quantitative estimations were carried out daily on the pooled sera of the four groups to determine the development of the following:—

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

1. *Agglutinins.*

The charts (Nos. 1–5) fully record, in the form of curves, the history of the development of these substances in the sera of the groups, and no protocols are therefore needed to supplement the information thus presented.

The technique described above proved satisfactory, and no difficulty was experienced in recording the results. The good emulsifying power of the "R" strain of typhoid simplified the work, and in all cases the control tubes, containing emulsion only, remained evenly turbid and checked the reading of the agglutination tubes.

Normal Limits of Agglutination.

In the early stages of the experiment the limit of normal agglutination was determined daily in each group. This was found to oscillate between a 1 in 4 and 1 in 10 dilution of serum. Inoculation had no immediate influence upon the amount of agglutinin normally present, and no noticeable changes were found until 9 days after inoculation.

First appearance of an increase in the Agglutinins.

In all four groups the rise commenced 9 days after inoculation, and it seems therefore as though dosage had little to do in hastening or retarding their appearance.

A parallel suggests itself between this first appearance of agglutinins after inoculation and the average date of their appearance in the blood in the course of an attack of enteric fever. Though it is a matter of difficulty to fix the latter point with certainty, the two periods evidently correspond closely.

Course of Development.

The charts speak for themselves in this repect, but it may be noted that we were somewhat taken by surprise at the rapidity of the rise in the agglutination value and the high levels ultimately attained, and on several occasions did not put up high enough dilutions to reach the end point; these occasions are recorded on the charts.

The very high levels attained by the sera of A, B, C groups will also be noted, and the influence of dosage on the levels attained and maintained is unmistakable.

It was thought well, in view of these high readings with a non-virulent strain to ascertain the agglutinating power of the sera upon a virulent strain of *Bacillus typhosus*, and this was kindly sent us by Dr. Martin. The virulence of this strain had been highly exalted by passage through guinea pigs, and it was lethal in 24 hours to a 250 gm. guinea pig in a dose of ·5 c.c. of a 24-hour broth culture. When tested by the same technique against the pooled sera, this virulent strain proved even more sensitive to the action of the agglutinins, the readings being higher in every case. The results are recorded separately in each chart.

Following on the rapid initial rise in the agglutinins a fall occurred in all the groups from 4 to 6 days after re-inoculation, and, following this, a second rise to a level higher than that previously attained.

This secondary rise commenced in all cases 9 days after re-inoculation, and this repetition of a 9-day interval between inoculation and a definite response in the elaboration of fresh agglutinins appears a fact of no little interest.

The very high level reached by group C (over 1–2000) is also remarkable, in view of the very small doses of vaccine given, ·1 c.c. and ·2 c.c.

A curious contrast is to be noticed in the effects of the first and second inoculations in A, B, C groups, the initial rise being greatest in C group and lowest in A, while the opposite is the case in the second rise after re-inoculation, the agglutinins here being in direct proportion to the dosage.

It will be of considerable interest to hear of the subsequent fate of these agglutinins in the various groups when they are followed up by Lieut. Smallman in India.

II. Bactericidal Substances.

The method of measuring these substances described above was adhered to throughout the course of the observations.

Plating out on the agar has the advantage over the broth method in that it affords evidence of the *degree* of bactericidal action in serum dilutions too weak to destroy the whole of the bacilli. It was thought, also, that the detection of contaminating organisms would have been facilitated, but, fortunately we had hardly any trouble of this sort.

The dilution of culture employed, 1 in 10,000, was, it should be noted, lower than that recommended by Dr. Wright, viz., 1 in 100,000. Previous experiment had shown us that the number of germs with the latter dilution of an average broth culture would only average about 20–30 in the volume employed in the tests and the lower dilution of 1 in 10,000, giving an average number of 200-300 germs, appeared to us to lessen the chance of errors due to large differences in dosage. This must therefore be borne in mind in contrasting our results with those previously recorded, inasmuch as the daily task set to the sera in the present experiment was a more severe one.

The two hours' interval which was always allowed between the collection of the blood and the drawing off of the serum from the clot was also fixed as the result of our preliminary work. The time the serum remains in contact with the clot was found to influence the bactericidal power to a very marked degree. Experiments were therefore conducted with a view to determining this point, and it was found that the maximum bactericidal effect was obtained in 2 hours, after which period no further rise could be detected by the method employed.

The period which has elapsed since the last meal is also apparently a factor to be reckoned with, but time did not admit of this point being worked out. It seems possible that this may be connected with the polynuclear leucocytosis occurring 2-3 hours after a meal. As the blood of the groups was, however, always collected at the same hour in the morning, after the men's breakfasts, this factor may safely be neglected in the present instance.

Influence of "Pooling" on the Bactericidal Power.

It was felt that it could not safely be taken for granted that the mixture of equal volumes of the serum of different individuals afforded an accurate measure of the average bactericidal power of a group of men. The following experiments were therefore carried out in which the individual values were obtained and the average of the results contrasted with that given by pooling the same sera.

Experiment I.

A.	Serum of A.B.S.	..	Killed in a dilution of	1	in	5
B.	„ D.H.	..	„	„	„	1	in	5
C.	„ F.T.	..	„	„	„	1	in	10
D.	„ W.B.L.	..	„	„	„	1	in	20
Pooled sera of A., B., C. and D.			„	„	„	1	in	10

Experiment II.

A.	Serum of F.T.	..	Killed in a dilution of	1	in	20
B.	„ D.H.	..	„	„	„	1	in	20
C.	„ W.B.L.	..	„	„	„	1	in	30
Pooled sera of A., B., and C.			„	„	„	1	in	20

We concluded from these experiments that "pooling" *does* afford an accurate means of estimating the average bactericidal power of a group of men.

Normal Limits of Bactericidal Power, as determined by the above method.

While the values registered for the various groups during the early days of the experiment appear to us to fix the average bactericidal power with a fair degree of accuracy, a number of individual observations were carried out on normal men, before, during, and after the Aldershot work, in order to determine as far as possible the limits of normal variation. In all, 21 separate observations were made, the technique described being adhered to in every case, while the dilutions of serum were the same as those employed in the main experiment.

The results were as follows:—

Sterility in	1- 5	dilution of serum,	4	times.
„	1-10	„	4	„
„	1-20	„	4	„
„	1-30	„	3	„
„	1-40	„	5	„
„	1-50	„	1	time.

There is thus seen to be a wide range of normal variations of bactericidal power, the lowest recorded being 1 in 5 diluted serum, and the highest, on only one occasion, 1 in 50 diluted serum, while the average of these 21 experiments is 1 in 23.

For pooled sera, such as we dealt with, the normal level of bactericidal power as tested by the above method may then be taken to lie between the dilutions of 1 in 20 and 1 in 30, and a line has been drawn in the charts to make this average normal value.

Explanation of the Protocols.

The results obtained in each serum dilution are recorded daily, the signs "0" signifying sterility, "+" growth of typhoid on the inoculated plate, and "—" that no experiment was made with that particular dilution.

In addition to recording "growth," the number of colonies that developed on the plate is recorded in brackets beside each "+" sign, and a careful consideration of these numbers will give a more accurate representation of the bactericidal power of the pooled serum on a given day than is to be obtained from a chart which must necessarily be plotted from an end point, arbitrarily selected.

A record has also been made each day of the results of the "count" of the broth culture employed, given in millions per c.c., and in a separate column, headed "Control," is given the number of colonies which developed on an agar plate from a volume of the 1 in 10,000 dilution of the daily broth culture approximately equal to the volume mixed in each tube with the diluted serum. This figure should be borne in mind in considering the results, as it represents the number of living typhoid germs with which each dilution of serum had to deal.

Explanation of the Charts. (*No.* 6.)

These are framed from the results recorded in detail in the protocols (Tables 1–4). The line drawn between the serum dilutions of 1 in 20 and 1 in 30 represents the average bactericidal power of the normal men whom we tested for this purpose.

In fixing the end point of each daily estimation for the purpose of record in form of a curve we have taken as evidence of a negative bactericidal effect the lowest dilution of serum in which two or more bacilli had survived. A consideration of the protocols will show that, in a considerable number of instances, a single bacillus has survived while in higher dilutions of serum the result is either complete sterility or, once more, a solitary survivor of the 200–300 germs introduced. In a certain number of instances the solitary colony may have been due to an accidental "splash" from another tube, made in blowing out the contents on to the agar plate (each plate serving for the testing of three tubes), but, in the majority, it would appear to be due to the fact that in every two or three hundred bacilli there are one or two

individuals endowed with a higher power of resistance to the bactericidal action of the serum than their neighbours. That degrees of resistance do occur among the bacilli is evident from the increasing number of survivors the higher the dilution of the serum.

It was thought that the exclusion of these single colonies would accordingly lead to a fairer representation of the actual power of the serum on a given day, and the dilution next below that from which two or more colonies developed was therefore adopted as the end point of sterility and the measure of the bactericidal power.

Should this system, however, be considered unjustifiable the necessary corrections of the charts can readily be made from the protocols.

In instances where irregular growths are recorded the cases were judged on their merits, and the system adopted was to ignore an irregular growth provided the higher dilutions gave evidence either of sterility or of the survival of only a single germ.

On one or two occasions the end point was not reached. Such observations are, of course, excluded from the charts, but shown in the protocols.

Commentary.

For six days after the inoculation there was no obvious change in the bactericidal power of any of the groups, and the values recorded are well within the limits of normal variation, as determined by the series of individual estimations recorded above. In no case was the end-point above 1 in 40 or below 1 in 20.

The first noticeable rise occurred on the seventh day in "B" and "D" groups; "A" group followed on the eighth day, and "C" on the ninth.

The subsequent course of the development in each group may be followed on the charts, and only special points will be referred to.

The Effects of the Second Dose upon the Bactericidal Curves.

At the time the re-inoculations were performed the bactericidal power was steadily rising in all the groups, and it will be seen that in "A," "B" and "C" groups this rise was in no degree checked but would rather appear to have been stimulated by the inoculation.

Whether the rise to the high levels attained during the two or three days following re-inoculation is to be attributed to the first or the second dose is a matter for conjecture, but at least it is obvious that no negative phase was the immediate result of re-inoculation with doses twice as large as those employed in the first instance.

In the case of group "D," a fall of one point lasting for two days was observed. As the second dose in this case was ten times larger than the first, this may possibly indicate a fall due to inefficient preparation by the very small dose first given—·01 c.c.

The Highest Points Attained.

In "A" and "B" the high level of 1 in 110 was reached, and in "C" group 1 in 90. In "D" group the figure 1 in 60 was never exceeded.

In all cases these maxima were reached on or before the third day after re-inoculation, and on the fourth day in all the groups a marked decline commenced, the high levels attained not being approached again. This fall in bactericidal power appeared to be interrupted by a partial recovery on the seventh day after re-inoculation, the same period, it may be noted, which elapsed between the primary inoculation and the first rise in bactericidal power.

On the eighth and ninth days after re-inoculation, a remarkable drop will be noticed in all the curves. As it was not anticipated the end point of sterility was not reached on the eighth day, but was found on the ninth day to be 1 in 10 in all the groups.

This heavy fall may, however, at least in part, be accounted for by the fact that, on these two days only, the broth used for diluting the culture was different to that which was ordinarily employed. It was noticed at the time to be darker in colour, and was only used owing to an accident to the reserve stock of the usual broth. Though there was none left to test, control experiments were subsequently carried out, using broth of varying degrees of alkalinity to dilute the same culture, and it was found that the reaction of the broth had a powerful effect in modifying the bactericidal power.

At the same time, although this may explain the very low values recorded on these two days, it is not impossible that the fall was a true one, and it has therefore been recorded in the curves. It may be noted, however, that the fall is as marked in "D" group after re-inoculation with ·1 c.c. of vaccine as in the other groups with larger doses, while no such fall followed the primary inoculation of group "C" with the same dose of ·1 c.c.

Whatever the true explanation of the fall, it was rapidly recovered from in all the groups.

At the last observations the bactericidal power of groups "A" and "B" was still considerably above the normal line, standing at 1 in 60 and 1 in 70 respectively, while in the case of "C" and "D" the values recorded had fallen within the normal limits of variation.

As in the case of the agglutinins, the further changes in the bactericidal power of the groups will be followed up by Lieutenant Smallman in India.

Bactericidal power of the sera tested upon a virulent strain of B. typhosus.

The culture obtained from the Lister Institute was again used for this purpose, and the results of the isolated experiments made are shown in the tabular form (Table 5), and may be contrasted with the results obtained on the same day with the non-virulent strain "R."

They have not been entered on the charts as the control experiments, made with the groups of normal sera, showed a lower average value than in the case of non-virulent culture.

In general, the values obtained were decidedly lower than those in the corresponding routine experiments which would appear to show that a virulent strain of *B. typhosus* is more resistant to the bactericidal action of the serum than the non-virulent strain employed for the inoculations.

Further, it would appear that the virulent strain is more uniformly resistant as the end-point of sterility is sharper and there are fewer examples of single germs surviving in stronger dilutions of serum.

III. Bacteriolysins.

The protocols (Tables 6–9) record the daily observations of the bacteriolytic power measured by the technique described above. The curves (Chart 7) have been plotted by taking as an end point the dilution of the serum in which all the bacilli had undergone spherulation and no unaltered rods were detected.

Normal bacteriolytic power.

As in the case of the bactericidal substances this was determined by a series of observations upon the serum of normal individuals and upon the pooled serum of normal men. The technique was of course the same as that used in the daily estimations. The results were very uniform, the end point being reached either with $\frac{2}{5}$ or $\frac{3}{5}$ diluted serum, no observations showing either a higher or a lower level than this.

A line representing the bacteriolytic power of normal serum has accordingly been drawn on each chart between the serum dilutions $\frac{3}{5}$ and $\frac{2}{5}$.

Commentary.

A rise in the bacteriolytic power followed inoculation in all the groups, and the degree and persistence of this rise was roughly proportionate to the dose of vaccine employed. The highest level, $\frac{1}{20}$, was attained by Group "A" after re-inoculation, and in Group "D" no higher value than $\frac{1}{5}$, or 1 point above the limits of normal variation, was recorded.

No evidence of a negative phase was manifested after re-inoculation.

In Group "A" it is of interest to note that the definite increase of of bacteriolysins on the 7th day after inoculation is reproduced 7 days after re-inoculation, while in the other groups with smaller doses of vaccine these substances appeared somewhat earlier—on the 5th or 6th day—though they did not subsequently reach such a high level as was attained by Group "A."

While the difficulties of an accurate classification of these results, to which reference has already been made, should be borne in mind, uniformity, at least, was secured by these estimations being always

made and recorded by the same observer. These difficulties were great, and it was regretted that time did not permit of further experiments directed to the elaboration of a more accurate method of measurement.

IV. Opsonins.

Although the results obtained are negative inasmuch as they fail to record the variations of the opsonins during immunization, they are embodied in the report (Chart 8, Table 10), since they serve to bring out some points of interest.

The results recorded by Wright and Douglas, although few in number, appeared to show that a definite opsonic effect was demonstrable upon typhoid bacilli, but it became evident, after our first observations upon the sera of the groups, that, if typhoid opsonins existed, their presence could not be demonstrated by the method we adopted. A reference to the protocols and charts will show that the results of the experiments were almost consistently in favour of the heated serum, *i.e.* that phagocytosis was higher in the case in which the serum had been heated to a temperature sufficient to destroy all active opsonin.

On noting this, a number of observations were made upon the sera of normal individuals, and it was found that, although occasionally a positive opsonic effect was apparent, in the great majority of cases a higher "phagocytic index" was obtained with "inactive" heated serum.

As the result of numerous experiments directed to the elucidation of this apparently contradictory result, we came to the conclusion that it was mainly accounted for by the bacteriolytic action of the unheated serum upon the typhoid bacilli. In the case of the tubes containing the unheated serum, mixed with the corpuscles and digested at blood heat for 15 minutes, a large proportion of the bacilli were destroyed by the serum and the phagocytes in consequence were provided with fewer opportunities of exercising their function than those in the corresponding tube containing the heated serum, in which bacteriolysis had not occurred to the same extent.

This bacteriolytic action of the serum was noted by Wright and Douglas, and pointed out as likely to mask opsonic action in the case of phagocytosis of typhoid bacilli, but, on the other hand, we have found such consistent and active phagocytosis of typhoid bacilli in the case of most normal heated sera, which contained, therefore, no active opsonin, that we were unable to convince ourselves of the existence of specific typhoid opsonins. At all events, from our experiments we are unable to endorse the opinion of Wright and Douglas that the typhoid bacillus is ' eminently susceptible" to the opsonic action of the blood fluids.

The daily experiments were, however, persevered in in the hopes, first, that an opsonic effect might manifest itself later as immunity became established, secondly, to see whether any correlation could be

observed between the degree of bacteriolysis, as recorded in the previous section, and the negative opsonic effects which resulted from our experiments with the pooled sera.

As no such evidence of increased opsonic effect or correlation with the bacteriolysins became manifest, the observations were discontinued four days after the re-inoculations.

Many attempts were made to obviate the fallacy of bacteriolysis by using heated emulsions, &c., but the results were irregular and unsatisfactory.

We can fully confirm all that Wright and Douglas say as to the alterations which take place in the bacilli, whether inside or outside the cells, in the case of the unheated serum, and the absence of these alterations in the case of the heated serum, but this appears to us simply an evidence of bacteriolysis and to afford no proof of an opsonic action of the serum.

Explanation of the Protocols and Charts.

The phagocytic index of each tube is recorded, and the ratio to 1 has been calculated between the indices of the heated and unheated serum tubes in each experiment. According as this ratio, obtained by dividing the higher index by the lesser, is in favour of the unheated or the heated serum, it is recorded above or below the central line of the chart which is marked "1" and signifies an identity in the indices of the two tubes.

Example:—

Heated Serum. Phagocytic Index = 20
Unheated „ „ „ = 10
Ratio in favour of Heated Serum = 2

V. Stimulins.

In adopting Metschnikoff's word "Stimulins" for the theoretical substances dealt with in this section, I must premise that I am not absolutely convinced of their identity. The experiments of Gengou, Klemperer, Besredka, and others alluded to by Metschnikoff, which attribute to certain normal and immune sera a stimulating action on the phagocytes of the animal into which they are injected, were all conducted upon living animals, and it is therefore difficult to compare their results with the experiments to which I have referred, which were conducted *in vitro*, either by my original method or by Wright and Douglas' modification. The stimulating effect of normal serum mentioned by them I have only been able to reproduce to a very slight degree by the methods indicated, and the stimulins to which this section refers appear to me to be specific in their action and peculiar to immune sera.

Whether they are identical or not, the stimulins of Metschnikoff, like those in question, were thermostable, withstanding the temperature of 60° C. without losing their stimulating properties, and thus in neither case can they be confused with the thermolabile opsonins.

Explanations of the Protocols and Charts. (*Chart* 9, *Table* 11.)

These have been constructed on the same lines as those dealing with the opsonic observations.

The Phagocytic Index of the control tube being ascertained, this was contrasted in each case with the indices of the tubes which contained a trace of the heated pooled serum of each group; the ratio was found and recorded as being either in favour of the control tube or in favour of the group serum tubes. Those ratios were taken to plot the curves recorded in the charts, where it will be noted the values above the central line —1— represent a positive stimulin effect, while those below line indicate a higher phagocytic power in the control tube.

At first, when little stimulin effect was anticipated, the volume of group serum added to the two volumes of heated normal serum, was undiluted, later, from the 4th November onwards, the volume was diluted 1–5 with normal salt solution to lessen the supposed effect of agglutination. The effect of the addition of this amount of salt solution was carefully tested by control experiments and was found to have no influence upon the results.

Living emulsions were employed except on the 10th November, when a heated culture was used.

Commentary.

The results obtained are sufficiently illustrated in the charts, and would seem to indicate a development of stimulins about the eleventh day. No marked contrasts are to be noted in the curves of the four groups, and the stimulating power of the sera did not appear to bear any relation to the dose of vaccine employed or to the amount of the agglutinins in the various groups. This latter fact I have also observed in connection with some of my former stimulin experiments.

PART IV.

General Commentary.

It should in the first place be borne in mind that the duration of the investigation only sufficed to trace the origin and development of the protective substances and the immediate effects of the first and second inoculations with different doses of vaccine. The further investigation of these substances is to be continued by Lieut. Smallman, in India, and his results will be communicated by me to the Committee from time to time.

1. *General Result of the Inoculations.*

It will be seen that, even with very small doses of vaccine, a remarkable development of protective substances occurred in the blood of the inoculated. At the conclusion of the investigation, four weeks after the first inoculations, the amount of these substances, in the majority of instances, remained considerably above the normal.

2. *The Local and General Re-actions following on Inoculation.*

In no case, even with the highest doses employed, did the reaction appear excessive. At the end of 48 hours all symptoms had disappeared, except in the case of a few individuals in whom pain and tenderness at the site of inoculation persisted for a day or two longer.

In general, the reactions were proportionate to the dosage employed.

A contrast was obtained in the case of Groups "A" and "B" as to the effect of the same dose of vaccine, ·6 c.c., employed in the case of "A," as a first dose, in the case of "B," as a second dose following a first inoculation of ·3 c.c. of vaccine. No marked differences were noticed in the character of the reaction.

3. *Effects of Dosage upon the Development of Protective Substances.*

The advantage appears distinctly to rest with Group "A," that which received the largest dose, while in the other groups the quantity of these substances developed bears a general relation to the quantity of vaccine employed.

This general relationship of protective substances to dosage of vaccine does not, however, appear to be in proportion to the differences in dosage: for instance, the values in Group "B" were only slightly lower than those recorded in Group "A," although "A" received twice as much vaccine as "B," and again, the quantity of protective substances developed in Group "C," who received but one-sixth of the dose given to "A," was remarkably high considering the small dose employed.

The result of the further investigations upon the blood of these groups in India, must be awaited before drawing conclusions from the persistence of these protective substances in the various groups as to the probable measure of protection afforded by the different doses.

4. *Question of the Development of a "Negative Phase."*

No evidence of the development of such a phase was found in any of the groups, either on first inoculation or re-inoculation. The system adopted of "pooling" the serum of the groups does not exclude the possibility of such a phase having developed in individual instances, but, had such been marked or common, our experiments should have given evidence of it, especially in the case of Group "A." The

further possibility of the negative phase being of a very transient character and thus escaping observation is theoretically possible, but it should be remembered that, in all cases, the blood was first tested within 16 hours after inoculation.

It seems probable, therefore, that, with dosage such as we employed, a negative phase, if developed, is so slight or so transient in nature as to be negligible.

5. *Interspacing of the Inoculations and Re-inoculations.*

The interval selected, of 11 days, between the first and second inoculations appears to be very suitable. At this time the protective substances formed in response to the first inoculation had made their appearance and were rapidly increasing, and re-inoculation appears to have stimulated rather than retarded their further elaboration.

6. *Results of Inoculation in Group "D."*

This group of older men, previously inoculated with typhoid vaccine 5 years ago, failed to show any very marked response to re-inoculation with ·01 c.c. of vaccine. They received, therefore, a ten-fold dose of ·1 c.c. as a second inoculation, but here too, as far as the experiment went, no unusual development of protective substances occurred.

Probably the interval of 5 years which in this case had elapsed since the former inoculation was too great.

7. *Value of the Agglutinin Curve as a Measure of the Protective Substances in General.*

The amount of agglutinins developed in the various groups appears to afford a general indication as to the development of the other protective substances, a fact which might perhaps be taken advantage of in future investigations in which it might not be practicable to carry out the more delicate technical processes.

(Signed) W. B. LEISHMAN,
Major R.A.M.C.

Pathological Laboratory,
Royal Army Medical College,
December 23rd, 1904.

TABLES AND CHARTS.

TABLE 1

A Group.—Bactericidal action of the pooled sera of 6 men, who received a dose of ·6 c.c. Anti-typhoid Vaccine on October 21st, and 1·2 c.c. on November 1st. Varying dilutions of the pooled sera mixed with equal volumes of 1–10,000 diluted 24-hour broth culture of Bacillus Typhosus (R.), incubated for 1 hour at 37° C., and then blown out on to Agar plates.

Date.	Count of culture per c.c.	Serum.												Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	1–80.	1–90.	1–100.	1–110.	1–120.	
Oct. 22nd	586 millions	0	0	0	+	+	+	—	—	—	—	—	—	—	Not counted.
„ 23rd	606 „	0	0	0	+ (1)	+ (3)	+ (5)	+ (29)	—	—	—	—	—	—	About 200 colonies.
„ 24th	486 „	0	+ (1)	0	0	+ (6)	+ (15)	+ (11)	—	—	—	—	—	—	About 150 colonies.
„ 25th	660 „	0	0	0	+ (2)	+ (3)	+ (4)	+ (10)	—	—	—	—	—	—	About 250 colonies.
„ 26th	600 „	—	0	0	0	+ (3)	+ (1)	+ (2)	+ (11)	—	—	—	—	—	About 200 colonies.
„ 27th	746 „	—	0	0	+ (6)	0	+ (1)	+ (4)	+ (1)	—	—	—	—	—	About 300 colonies.
„ 28th	640 „	0	0	0	+ (2)	+ (1)	— (plate dried)	— (plate dried)	—	—	—	—	—	—	About 250 colonies.
„ 29th	600 „	—	0	—	0	0	0	+ (3)	+	+ (15)	+ (47)	+ (60)	—	—	About 200 colonies.
„ 30th	646 „	—	—	—	0	0	0	+ (1)	+ (6)	+ (24)	+ (30)	+ (55)	—	—	About 200 colonies.
„ 31st	750 „	—	—	—	0	0	+ (2)	0	+ (2)	+ (7)	—	—	—	—	About 250 colonies.

Nov. 1st	634 „	–	–	–	–	+ (1)	0	0	0	+ (2)	–	–	–	–	About 150 colonies.
„ 2nd	780 „	–	–	0	0	0	0	0	0	0	0	–	–	–	About 250 colonies.
„ 3rd	800 „	–	–	0	0	0	0	0	0	0	0	+ (1)	0	+ (20)	About 300 colonies.
„ 4th	826 „	–	–	–	–	–	–	0	0	0	+ (1)	+ (1)	+ (1)	+ (2)	About 300 colonies.
„ 5th	814 „	–	–	–	–	–	–	–	0	0	+ (3)	+ (11)	+ (19)	+ (58)	About 300 colonies.
„ 6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
„ 7th	926 millions	–	–	–	–	–	–	0	+ (3)	+ (9)	+ (16)	+ (42)	+ (57)	+ (about 100)	About 350 colonies.
„ 8th	886 „	–	–	–	–	–	0	0	0	+ (12)	+ (9)	+ (34)	–	–	About 350 colonies.
„ 9th	700 „	–	–	–	–	–	+ (11)	+ (40)	+ (40)	+ (about 100)	+ (about 100)	+ (about 200)	–	–	About 300 colonies.
„ 10th	616 „	–	0	+ (5)	+ (22)	+ (about 50)	+ (about 70)	+ (about 50)	+ (about 70)	–	–	–	–	–	About 250 colonies.
„ 11th	906 „	–	0	0	0	+ (2)	+ (1)	–	–	–	–	–	–	–	About 350 colonies.
„ 12th	780 „	–	0	0	0	0	+ (1)	0	+ (1)	+ (1)	+ (3)	–	–	–	About 300 colonies.
„ 13th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
„ 14th	960 millions	–	0	0	0	+ (1)	+ (4)	+ (4)	0	+ (20)	+ (18)	+ (about 150)	–	–	About 450 colonies.
„ 15th	704 „	–	0	–	+ (2)	0	0	+ (2)	+ (4)	+ (8)	+ (13)	+ (37)	–	–	About 300 colonies.
„ 16th	740 „	–	–	–	0	+ (1)	+ (1)	0	+ (4)	+ (10)	+ (17)	–	–	–	About 300 colonies.

+ = Growth; numbers where given are the number of colonies which grew. 0 = Sterile. – = Not tried.

TABLE 2.

B Group.—Bactericidal action of the pooled sera of 8 men, who received a dose of ·3 c.c. Anti-typhoid Vaccine on October 21st and ·6 c.c. on November 1st. Varying dilutions of the pooled sera mixed with equal volumes of a 1–10,000 diluted 24-hour broth culture of Bacillus Typhosus (R.), incubated for 1 hour at 37° C. and then blown out on to Agar plates.

Date.	Count of culture per c.c.	Serum.												Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	1–80.	1–90.	1–100.	1–110.	1–120.	
Oct. 22nd	586 millions	0	0	0	0	+ (14)	+ (11)	–	–	–	–	–	–	–	Not counted.
,, 23rd	606 ,,	0	0	0	+ (2)	+ (12)	+ (15)	+	–	–	–	–	–	–	About 200 colonies.
,, 24th	486 ,,	0	0	0	0	+ (2)	+ (3)	+ (6)	–	–	–	–	–	–	About 150 colonies.
,, 25th	660 ,,	0	0	0	0	0	+ (3)	+ (15)	–	–	–	–	–	–	About 250 colonies.
,, 26th	600 ,,	0	0	0	0	+ (2)	+ (2)	+ (10)	–	–	–	–	–	–	About 200 colonies.
,, 27th	746 ,,	0	0	0	0	+ (2)	+ (1)	+ (1)	–	–	–	–	–	–	About 300 colonies.
,, 28th	640 ,,	0	0	0	+ (1)	0	0	+ (2)	–	–	–	–	–	–	About 250 colonies.
,, 29th	600 ,,	–	–	–	0	0	0	+ (5)	+ (5)	+ (7)	+ (15)	+ (25)	–	–	About 200 colonies.
,, 30th	646 ,,	–	–	–	0	0	0	+ (3)	+ (5)	+ (8)	+ (24)	(45)	–	–	About 200 colonies.
,, 31st	750 ,,	–	0	–	0	0	0	+ (2)	+ (2)	+ (4)	–	–	–	–	About 250 colonies.

Nov. 1st	634 ,,	–	–	–	–	0	0	+ (1)	+ (1)	+ (5)	+ (9)	–	–	–	About 150 colonies.
,, 2nd	780 ,,	–	–	0	0	0	0	0	+ (1)	+ (1)	+ (about 80)	–	–	–	About 250 colonies.
,, 3rd	800 ,,	–	–	0	0	0	0	0	0	0	+ (1)	+ (4)	+ (2)	+ (about 50)	About 300 colonies.
,, 4th	826 ,,	–	–	–	–	–	–	0	0	0	0	0	0	+ (2)	About 300 colonies.
,, 5th	814 ,,	–	–	–	–	–	–	–	0	+ (4)	+ (4)	+ (8)	+ (12)	+ (about 100)	About 300 colonies.
6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
,, 7th	926 millions	–	–	–	–	0	0	+ (2)	+ (12)	+ (18)	+ (25)	+ (21)	+ (58)	–	About 350 colonies.
,, 8th	886 ,,	–	–	–	0	0	+ (1)	+ (1)	+ (4)	+ (10)	+ (5)	–	–	–	About 350 colonies.
,, 9th	700 ,,	–	–	–	+ (about 40)	+ (14)	+ (21)	0	+ (abou 50)	+ (about 70)	–	–	–	–	About 300 colonies.
,, 10th	616 ,,	–	0	+ (6)	+ (38)	+ (56)	+ (42)	+ (46)	+ (60)	–	–	–	–	–	About 250 colonies.
,, 11th	906 ,,	–	0	0	0	0	0	0	–	–	–	–	–	–	About 350 colonies.
,, 12th	780 ,,	–	0	0	0	0	0	+ (1)	+ (4)	+ (14)	+ (12)	–	–	–	About 300 colonies.
,, 13th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
,, 14th	960 millions	–	0	0	0	0	+ (1)	+ (2)	0	+ (2)	+ (8)	+ (17)	–	–	About 450 colonies.
,, 15th	704 ,,	–	0	–	0	0	+ (8)	+ (1)	+ (3)	+ (13)	+ (35)	+ (57)	–	–	About 300 colonies.
,, 16th	7.0 ,,	–	–	0	0	0	+ (1)	+ (1)	+ (1)	+ (5)	+ (13)	–	–	–	About 300 colonies.

+ = Growth; numbers where given are the number of colonies which grew. 0 = Sterile. – = Not tried.

TABLE 3.

C Group.—Bactericidal action of the pooled sera of 6 boys, who received a dose of ·1 c.c. Anti-typhoid Vaccine on October 21st, and ·2 c.c. on November 1st. Varying dilutions of the pooled sera mixed with equal volumes of a 1–10,000 diluted 24-hour broth culture of Bacillus Typhosus (R.), incubated for 1 hour at 37° C., and then blown out on to Agar plates.

Date.	Count of culture per c.c.	Serum.												Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	1–80.	1–90.	1–100.	1–110.	1–120.	
Oct. 22nd	586 millions	0	0	0	+ (1)	0	+ (2)	—	—	—	—	—	—	—	Not counted.
,, 23rd	606 ,,	0	0	0	0	+ (3)	+ (6)	+ (17)	—	—	—	—	—	—	About 200 colonies.
,, 24th	486 ,,	0	0	0	0	+ (2)	+ (3)	+ (many)	—	—	—	—	—	—	About 150 colonies.
,, 25th	660 ,,	0	0	0	+ (2)	0	0	+ (many)	—	—	—	—	—	—	About 250 colonies.
,, 26th	600 ,,	0	0	0	0	0	+ (4)	0	—	—	—	—	—	—	About 200 colonies.
,, 27th	746 ,,	0	+ (1)	0	0	0	+ (6)	+ (6)	0	—	—	—	—	—	About 300 colonies.
,, 28th	640 ,,	0	0	0	0	0	+ (3)	+ (2)	+ (3)	—	—	—	—	—	About 250 colonies.
,, 29th	600 ,,	—	—	—	—	+ (2)	+ (1)	+ (2)	+ (1)	+ (2)	+ (26)	+ (26)	—	—	About 200 colonies.
,, 30th	646 ,,	—	—	—	+ (1)	0	0	+ (38)	+ (9)	+ (26)	+ (47)	+ (about 100)	—	—	About 200 colonies.
,, 31st	750 ,,	—	—	—	0	0	+ (1)	+ (2)	+ (4)	+ (4)	+ (13)	—	—	—	About 250 colonies.

Nov. 1st	634 „	–	–	–	–	+ (1)	0	0	0	+ (8)	+ (8)	–	–	–	About 150 colonies.
„ 2nd	780 „	–	–	0	0	0	0	0	+ (1)	0	0	–	–	–	About 250 colonies.
„ 3rd	800 „	–	–	0	0	0	0	0	+ (1)	+ (2)	+ (8)	+ (40)	+ (48)	+ (65)	About 300 colonies.
4th	826 „	–	–	–	–	–	0	0	0	0	0	+ (5)	+ (3)	+ (3)	About 300 colonies.
„ 5th	814 „	–	–	–	–	–	–	0	+ (2)	+ (13)	+ (20)	+ (41)	+ (about 50)	+ (about 70)	About 300 colonies.
„ 6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
„ 7th	926 millions	–	–	–	–	0	+ (2)	+ (1)	+ (2)	+ (19)	+ (36)	+ (67)	–	–	About 350 colonies.
„ 8th	886 „	–	0	0	0	0	+ (1)	+ (1)	+ (1)	+ (3)	–	–	–	–	About 350 colonies.
„ 9th	700 „	–	–	+ (2)	+ (9)	+ (18)	+ (about 50)	+ (about 70)	+ (about 100)	+ (10)	–	–	–	–	About 300 colonies.
„ 10th	616 „	–	0	+ (5)	+ (31)	+ (32)	+ (about 50)	+ (about 60)	+ (about 70)	–	–	–	–	–	About 250 colonies.
„ 11th	906 „	–	0	0	0	0	+ (1)	0	–	–	–	–	–	–	About 350 colonies.
„ 12th	780 „	–	0	0	0	0	+ (1)	0	0	+ (2)	–	–	–	–	About 300 colonies.
„ 13th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
„ 14th	960 millions	5 members of group absent, experiment omitted.													About 450 colonies.
„ 15th	704 „	–	0	0	0	0	0	+ (5)	–	+ (43)	–	+ (about 100)	–	–	About 300 colonies.
„ 16th	740 „	–	0	+ (1)	+ (1)	+ (2)	+ (2)	0	+ (6)	+ (1)	–	–	–	–	About 300 colonies.

+ = Growth; numbers where given are the number of colonies which grew. 0 = Sterile. – = Not tried.

TABLE 4.

D Group.—Bactericidal action of the pooled sera of 5 men who had been inoculated against Typhoid Fever 5 years previously, and who received a dose of ·01 c.c. Anti-typhoid Vaccine on October 21st and ·1 c.c. on November 1st. Varying dilutions of the pooled sera mixed with equal volumes of a 1–10,000 diluted 24-hour broth culture of Bacillus Typhosus (R.), incubated for 1 hour at 37° C. and then blown out on to Agar plates.

Date.	Count of culture per c.c.	Serum.												Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	1–80.	1–90.	1–100.	1–110.	1–120.	
Oct. 22nd	586 millions	0	0	0	+ (1)	+ (4)	+ (12)	–	–	–	–	–	–	–	Not counted.
,, 23rd	606 ,,	0	0	0	+	+	+	+	–	–	–	–	–	–	About 200 colonies.
,, 24th	486 ,,	0	0	0	+ (2)	+ (15)	+ (6)	+ (23)	–	–	–	–	–	–	About 150 colonies.
5th	660 ,,	0	0	0	0	0	+ (3)	+ (4)	–	–	–	–	–	–	About 250 colonies.
,, 26th	600 ,,	0	0	0	0	+ (3)	+ (25)	+ (5)	–	–	–	–	–	–	About 200 colonies.
,, 27th	746 ,,	0	0	0	+ (4)	+ (2)	+ (3)	+ (2)	+ (11)	–	–	–	–	–	About 300 colonies.
,, 28th	640 ,,	–	0	0	0	0	0	+ (3)	–	–	–	–	–	–	About 250 colonies.
,, 29th	600 ,,	–	–	–	0	+ (1)	0	+ (4)	+ (12)	+ (37)	+ (30)	+ (about 150)	–	–	About 200 colonies.
,, 30th	646 ,,	–	–	–	0	0	0	+ (7)	+ (6)	+ (36)	+ (28)	+ (about 150)	–	–	About 200 colonies.
,, 31st	750 ,,	–	–	–	–	0	0	0	+ (3)	+ (36)	+ (71)	–	–	–	About 250 colonies.

Nov. 1st	634 „	–	–	–	–	0	0	0	+ (8)	+ (20)	+ (36)	–	–	–	About 150 colonies.
„ 2nd	780 „	–	–	0	0	0	0	+ (5)	0	+ (about 100)	+ (13)	–	–	–	About 250 colonies.
„ 3rd	800 „	–	–	0	0	0	+ (1)	+ (3)	+ (37)	+ (about 50)	+ (37)	+ (about 100)	+ (about 100)	+ about 150)	About 300 colonies.
„ 4th	826 „	–	–	–	0	0	0	+ (1)	+ (4)	+ (2)	–	–	–	–	About 300 colonies.
„ 5th	814 „	–	–	–	0	+ (5)	+ (31)	+ (about 60)	+ (about 60)	+ (about 100)	–	–	–	–	About 300 colonies.
„ 6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
„ 7th	926 millions	–	–	0	0	0	+ (5)	+ (14)	+ (15)	+ (60)	–	–	–	–	About 350 colonies.
„ 8th	886 „	–	–	0	0	0	0	+ (8)	+ (about 80)	+ (about 80)	–	–	–	–	About 350 colonies.
„ 9th	700 „	–	–	+ (7)	+ (about 50)	+ (about 100)	+ (about 100)	+ (about 150)	+ (25)	+ (about 200)	–	–	–	–	About 300 colonies.
„ 10th	616 „	–	0	+ (8)	+ (35)	+ (9)	–	+ (about 50)	–	–	–	–	–	–	About 250 colonies.
„ 11th	906 „	–	0	0	+ (1)	+ (11)	–	+ (25)	–	–	–	–	–	–	About 350 colonies.
„ 12th	780 „	–	0	0	0	+ (5)	+ (9)	+ (2)	+ (46)	+ (about 60)	–	–	–	–	About 300 colonies.
„ 13th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
„ 14th	960 millions					4 members of group absent, experiment omitted.									–
„ 15th	704 „					3 members of group absent, experiment omitted.									–
„ 16th	740 „	–	0	0	0	+ (1)	+ (9)	+ (41)	+ (42)	+ (33)	–	–	–	–	About 300 colonies.

+ = Growth numbers where given are the number of colonies which grew. 0 = Sterile. – = Not tried.

TABLE 5.

I.—November 8th, 1904.—**Bactericidal action** of A and B Group Sera on a virulent Bacillus Typhosus compared with that on Bacillus Typhosus (R). The virulent culture was one the fatal dose of which for a 250 grm. guinea pig in 24 hours was 0·5 c.c. of a 24-hour broth culture.

	Virulent Bacillus Typhosus.—Count = 480 millions per c.c.						Bacillus Typhosus (R.).—Count = 886 millions per c.c.					
	Serum.					Control.	Serum.					Control.
	1–20.	1–30.	1–40.	1–50.	1–60.		1–20.	1–30.	1–40.	1–50.	1–60.	
A	0	+ (40)	+ (42)	+ (about 200)	+ (about 200)	About 300 colonies.	—	—	—	0	0	About 350 colonies.
B	0	+ (51)	+ (about 80)	+ (about 100)	+ (about 200)		—	0	0	+ (1)	+ (1)	

II.—November 9th, 1904.—**Bactericidal action** of A and B Group Sera on the same virulent Bacillus Typhosus, compared with that of the pooled sera of 3 normal men.

Count = 286 millions per c.c.

	Serum.							Control.
	1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	
A	—	0	+ (6)	+ (6)	+ (22)	+ (25)	+ (about 50)	About 200 colonies.
B	—	0	0	+ (1)	+ (2)	+ (13)	+ (about 30)	
Normal	+ (1)	+ (3)	+ (19)	+ (35)	+ (about 50)	+ (about 50)	+ (about 70)	

III.—November 11th, 1904.—**Bactericidal action** of A, B, C and D Group Sera on the same virulent culture, compared with that of the pooled sera of 6 normal men.

Count = 460 millions per c.c.

	Serum.							Control.
	1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	
A	0	+ (1)	+ (7)	+ (about 50)	+ (about 100)	+ (about 150)	–	About 300 colonies.
B	0	0	0	+ (58)	+ (54)	–	+ (about 100)	
C	0	0	0	0	+ (31)	+ (about 150)	–	
D	0	0	+ (2)	+ (50)	+ (50)	+ (about 150)	–	
Normal	0	0	+ (52)	+ (about 70)	+ (about 150)	(+ about 200)	–	

IV.—December 6th, 1904.—**Bactericidal action** of the serum of a normal man on the same virulent culture, compared with that on Bacillus Typhosus (R.).

Culture.	Count.	Serum.								Control.
		1–5.	1–10.	1–20.	1–30.	1–40.	1–50.	1–60.	1–70.	
Bacillus Typhosus (R.)	126 millions ..	0	0	0	0	0	+ (2)	+ (1)	+ (8)	About 150 colonies.
Bacillus Typhosus (virulent) ..	266 „ ..	0	0	0	+ (9)	+ (4)	+ (118)	+ (113)	+ (128)	About 200 colonies.

TABLE 6.

A Group.—Bacteriolytic action of the pooled sera of 6 men who received a dose of ·6 c.c. Anti-typhoid Vaccine on October 21st, and 1·2 c.c. on November 1st. Equal volumes of a 24-hour broth culture of Bacillus Typhosus (R.) and of varying dilutions of the serum were mixed, incubated for 1 hour at 37° C., then blown out on to slides and stained with methylene blue.

Date.	Serum.						
	Undiluted.	4–5.	3–5.	2–5.	1–5.	1–10.	1–20.
*October 22nd .. .	+	−	−	−	−		
* „ 23rd	+	+	+	+	−	−	
„ 24th			Not	tried.			
„ 25th	+	+	±	−	−	−	
„ 26th	+	+	+	±	−	−	
„ 27th			+	±	−	−	0
„ 28th			+	+	+	±	−
„ 29th			+	+	±	−	0
„ 30th			+	+	±	−	−
„ 31st			+	+	±	−	0
November 1st			Not	tried.			
„ 2nd			+	+	±	±	−
„ 3rd			+	+	+	±	−
„ 4th			Not	tried.			
„ 5th			+	+	±	±	−
„ 6th			Not	tried.			
„ 7th			+	±	±	±	−
„ 8th			+	+	±	±	±
„ 9th			Not	tried.			
„ 10th			+	±	±	−	−
„ 11th			Not	tried.			
„ 12th			+	+	+	±	−
„ 13th			Not	tried.			
„ 14th			+	+	+	±	−
„ 15th			Not	tried.			
„ 16th			+	+	±	±	−

+ = Complete bacteriolysis. − = Spherulated and unaltered bacteria present.
± = All bacteria spherulated. 0 = No appreciable bacteriolysis.
* The standard taken for these two days was presence (−) or absence (+) of recognisable bacteria.

TABLE 7.

B Group.—Bacteriolytic action of the pooled sera of 8 men who received a dose of ·3 c.c. Anti-typhoid Vaccine on October 21st, and ·6 c.c. on November 1st. Equal volumes of a 24-hour broth culture of Bacillus Typhosus (R.) and of varying dilutions of the serum were mixed, incubated for 1 hour at 37° C., then blown out on to slides and stained with methylene blue.

Date.	Serum.						
	Undiluted.	4–5.	3–5.	2–5.	1–5.	1–10.	1–20.
*October 22nd	+	+	+	−	−	−	
* „ 23rd	+	+	−	+	−	−	
„ 24th			Not	tried.			
„ 25th	+	+	+	±	−	0	
„ 26th	+	+	+	+	±	−	
„ 27th			+	±	−	−	0
„ 28th			+	±	±	±	−
„ 29th			+	+	+	±	−
„ 30th			+	+	±	±	−
„ 31st			+	±	±	±	−
November 1st			Not	tried.			
„ 2nd			+	+	±	±	−
„ 3rd			+	+	±	±	−
„ 4th			Not	tried.			
„ 5th			±	±	±	−	0
„ 6th			Not	tried.			
„ 7th			+	+	±	±	−
„ 8th			+	+	±	±	−
„ 9th			Not	tried.			
„ 10th			+	+	±	±	−
„ 11th			Not	tried.			
„ 12th			+	+	+	±	−
„ 13th			Not	tried.			
„ 14th			+	+	±	±	−
„ 15th			Not	tried.			
„ 16th			+	±	±	−	0

+ = Complete bacteriolysis. − = Spherulated and unaltered bacteria present.
± = All bacteria spherulated. 0 = No appreciable bacteriolysis.
* The standard taken on these two days was the presence (−) or absence (+) of recognisable bacteria.

TABLE 8.

C Group.—Bacteriolytic action of the pooled sera of 6 "boys" who received a dose of ·1 c.c. Anti-typhoid Vaccine on October 21st, and ·2 c.c. on November 1st. Equal volumes of a 24-hour broth culture of Bacillus Typhosus (R.), and of varying dilutions of the serum were mixed, incubated for 1 hour at 37° C., then blown out on to slides and stained with methylene blue.

Date.	Serum.						
	Undiluted.	4–5.	3–5.	2–5.	1–5.	1–10.	1–20.
*October 22nd	+	+	+	−	−		
* „ 23rd	+	+	+	−	+	−	
„ 24th			Not	tried.			
„ 25th	+	+	±	−	0	0	
„ 26th	+	+	+	±	±	±	
„ 27th			+	+	±	±	−
„ 28th			+	+	±	−	0
„ 29th			+	+	±	−	−
„ 30th			+	±	−	0	0
„ 31st			+	+	±	±	−
November 1st			+	+	±	−	−
„ 2nd			+	+	±	−	−
„ 3rd			+	+	±	−	−
„ 4th			Not	tried.			
„ 5th			±	±	±	−	0
„ 6th			Not	tried.			
„ 7th			+	±	±	−	−
„ 8th			+	±	−	−	−
„ 9th			Not	tried.			
„ 10th			±	±	±	−	0
„ 11th			Not	tried.			
„ 12th			+	+	±	±	−
„ 13th			Not	tried.			
„ 14th			Not	tried.			
„ 15th			+	±	±	±	−
„ 16th			Not	tried.			

\+ = Complete bacteriolysis. − = Spherulated and unaltered bacteria present.
± = All bacteria spherulated. 0 = No appreciable bacteriolysis.
* The standard taken on these days was the presence (−) or the absence (+) of recognisable bacteria.

TABLE 9.

D Group.—Bacteriolytic action of the pooled sera of 5 men who had been inoculated against Typhoid Fever 5 years previously, and who received a dose of ·01 c.c. Anti-typhoid Vaccine on October 21st, and ·1 c.c. on November 1st. Equal volumes of a 24-hour broth culture of Bacillus Typhosus (R.) and of varying dilutions of the serum were mixed, incubated for 1 hour at 37° C., then blown out on to slides and stained with methylene blue.

Date.	Serum.						
	Undiluted.	4–5.	3–5.	2–5.	1–5.	1–10.	1–20.
*October 22nd	+	+	+	−	−		
* ,, 23rd	+	+	−	+	+	−	
,, 24th			Not	tried.			
,, 25th	+	+	±	−	−	0	
,, 26th	+	+	+	±	−	−	
,, 27th			+	±	±	−	−
,, 28th			±	±	±	−	0
,, 29th			±	±	−	−	0
,, 30th			±	±	−	0	0
,, 31st			+	±	±	−	0
November 1st			Not	tried.			
,, 2nd			+	+	±	−	0
,, 3rd			+	+	±	−	−
,, 4th			Not	tried.			
,, 5th			±	±	−	−	0
,, 6th			Not	tried.			
,, 7th			±	±	−	−	0
,, 8th			+	±	−	−	0
,, 9th			Not	tried.			
,, 10th			±	−	−	−	0
,, 11th			Not	tried.			
,, 12th			+	+	±	−	0
,, 13th			Not	tried.			
,, 14th			Not	tried.			
,, 15th			Not	tried.			
,, 16th			±	±	−	−	0

+ = Complete bacteriolysis. − = Spherulated and unaltered bacteria present.
± = All bacteria spherulated. 0 = No appreciable bacteriolysis.
* The standard taken on these two days was the presence (−) or the absence (+) of recognisable bacteria.

TABLE 10.

"OPSONINS."

Date.	Group "A."				Group "B."				Group "C."				Group "D."			
	Phagocytic Index		Ratio in favour		Phagocytic Index		Ratio in favour		Phagocytic Index		Ratio in favour		Phagocytic Index		Ratio in favour	
	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.	Of unheated Serum.	Of heated Serum.
October 22nd	6·1	5·7	1·06	—	4·1	7·7	—	1·88	5·2	7·2	—	1·38	5·4	11·5	—	2·12
„ 23rd	10·3	3·6	2·95	—	7	5·1	1·37	—	8·9	8·6	1·03	—	10·5	8·5	1·23	—
„ 24th	6·7	5·1	1·3	—	7·4	10·6	—	1·52	7	5	1·4	—	3·7	14·5	—	3·9
„ 25th	5·9	4·8	1·23	—	4·3	7·2	—	1·67	5·2	7	—	1·35	6	8·1	—	1·35
„ 26th	2·1	9·3	—	4·4	5	8·8	—	1·76	4·35	11·8	—	2·66	4·15	12·15	—	2·92

„ 27th	5·64	7·76	—	1·37	3	12	—	4	4·4	7·52	—	1·7	4·32	11·16	—	2·56
„ 28th	4·8	8·6	—	1·8	3·7	10·5	—	2·85	6·2	4·4	1·4	—	3·5	3·4	1·03	—
„ 29th	3·8	3·8	1	1	3·56	6·28	—	1·76	4·4	11·28	—	2·56	4·4	3·96	1·11	—
„ 30th	7·7	17·3	—	2·25	8·15	10·6	—	1·37	10·2	8	1·27	—	8	16·2	—	2·02
November 1st	1·7	3·6	—	2·1	2·2	2·8	—	1·27	7	30·6	—	4·35	4·8	25·3	—	5·25
„ 2nd .. .	2·3	3·5	—	1·52	4·5	9·7	—	2·15	5·4	4·5	1·2	—	3·9	7·2	—	1·85
„ 3rd	3·76	9·48	—	2·5					5·5	13·4	—	2·42	4·3	4·8	—	1·11
„ 4th	2·5	1·5	1·66	—	2·5	7·2	—	2·87	2·6	8·2	—	3·15				

TABLE 11.

"STIMULINS."

Date.	Control.	Group "A."			Group "B."			Group "C."			Group "D."		
	Phagocytic Index.	Phagocytic Index.	Ratio in favour of		Phagocytic Index.	Ratio in favour of		Phagocytic Index.	Ratio in favour of		Phagocytic Index.	Ratio in favour of	
			Control.	Group Serum.		Control.	Group Serum.		Control.	Group Serum.		Control.	Group Serum.
October 22nd ..	15	14·6	1·02	–	12·4	1·2	–	16	–	1·06	14·1	1·06	–
„ 23rd ..	4	4·5	–	1·13	4·2	–	1·03	5·9	–	1·47	4·9	–	1·23
„ 24th ..	15·8	11·6	1·36	–	19·6	–	1·24	9	1·75	–	15·9	1	1
„ 25th ..	13·45	12·7	1·05	–	14·7	–	1·09	11	1·21	–	9·3	1·44	–
„ 26th ..	21	16·2	1·31	–	13·1	1·63	–	12·5	1·7	–	14·5	1·46	–

„ 27th	..	13·08	9·56	1·37	–	18·04	–	1·38	14·8	–	1·13	12·6	1·03	–
„ 28th	..	15·7	16·1	–	1·01	15·8	–	1·01	10·7	1·46	–	11·6	1·35	–
„ 29th	..	12·92	10·28	1·25	–	13·56	–	1·04	14·8	–	1·14			
„ 30th	..	15·7	24·7	–	1·58	16·5	–	1·05	11	1·43	–	10·8	1·46	–
November 1st	..	5·2	4·1	1·27	–	11	–	2·1	12	–	2·3	15·5	–	2·98
„ 2nd	..	8·5	16·7	–	1·95	9·5	–	1·11	17	–	2	11·3	–	1·33
„ 3rd	..	1·7				2·4	–	1·41						
„ 4th	..	1	9	–	9	7·2	–	7·2	3·3	–	3·3	5·1	–	5·1
„ 5th	..	·6	1·4	–	2·33	1·2	–	2	1·36	–	2·26	·8	–	1·33
„ 7th	..	·65	3·4	–	5·2	1·5	–	2·3	1·8	–	2·76	2·6	–	4
„ 10th	..	2·6	2	1·3	–	3·4	–	1·3	4	–	1·54	2·7	–	1·01
„ 12th	..	2	2·6	–	1·3	3	–	1·5	4·5	–	2·25	2·3	–	1·15

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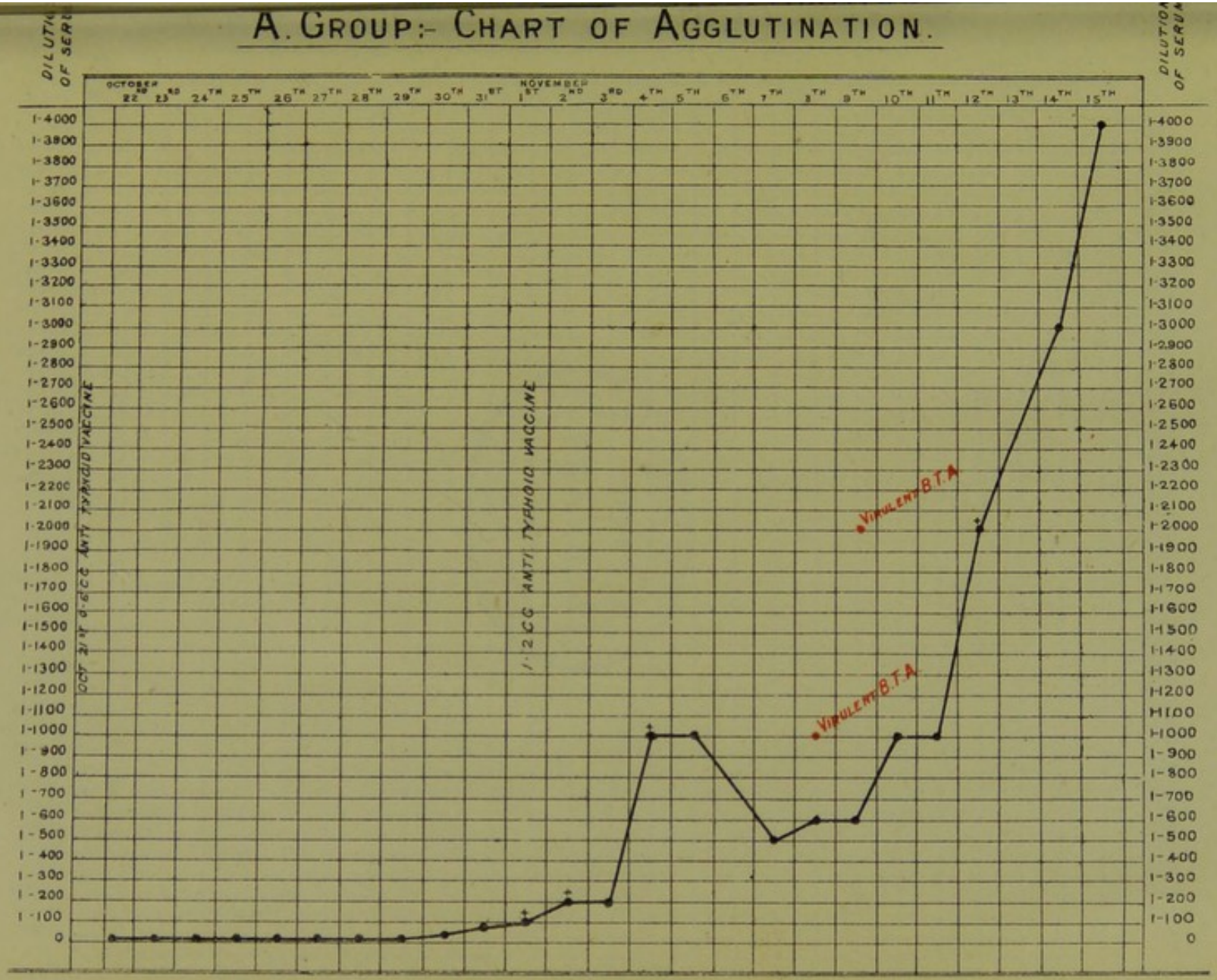
A. GROUP:- CHART OF AGGLUTINATION.

CHART. I.



+ = HIGHEST DILUTION OF THE SERUM TRIED.

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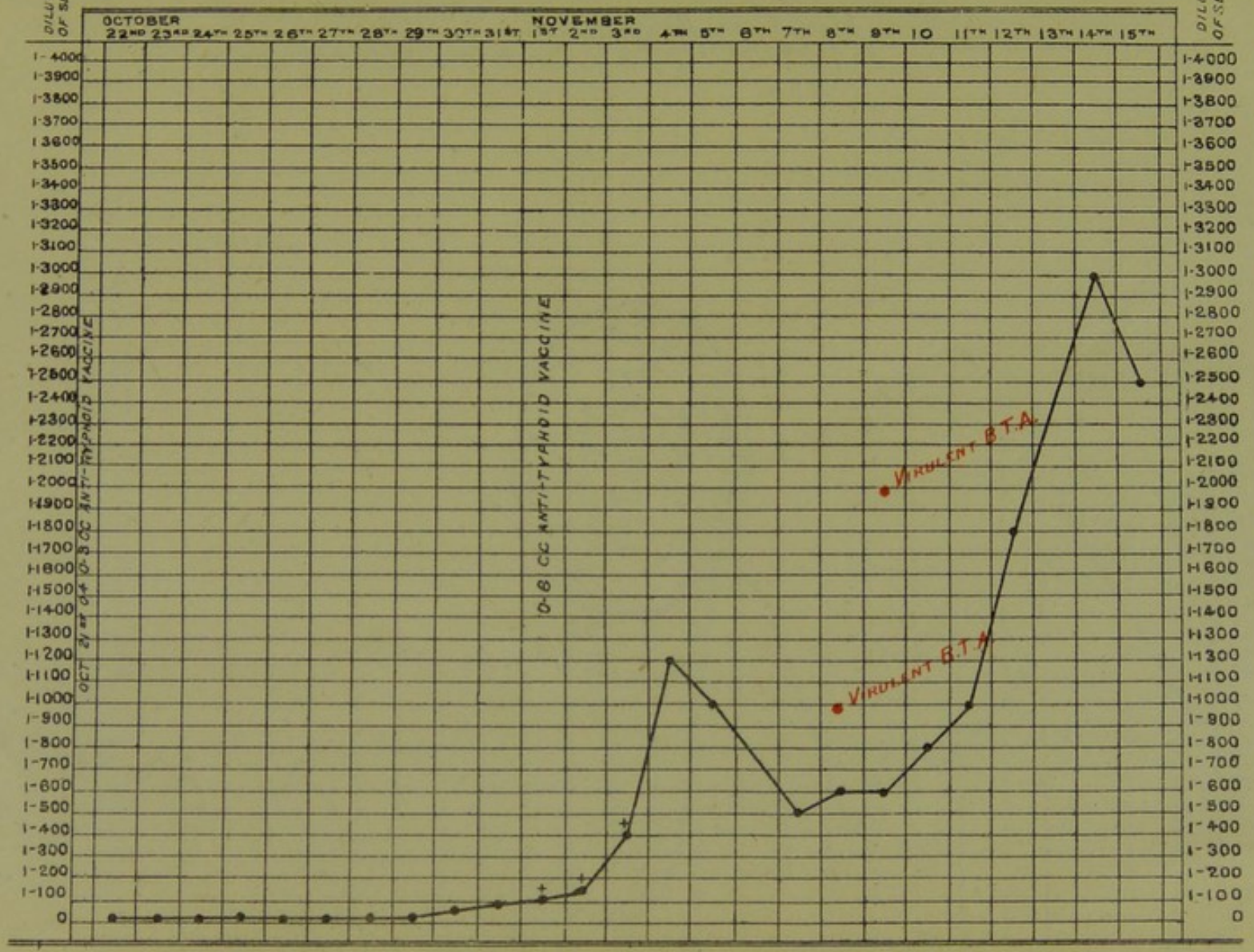
B. GROUP :- CHART OF AGGLUTINATION.

CHART 2



\+ = HIGHEST DILUTION OF THE SERUM TRIED

5446

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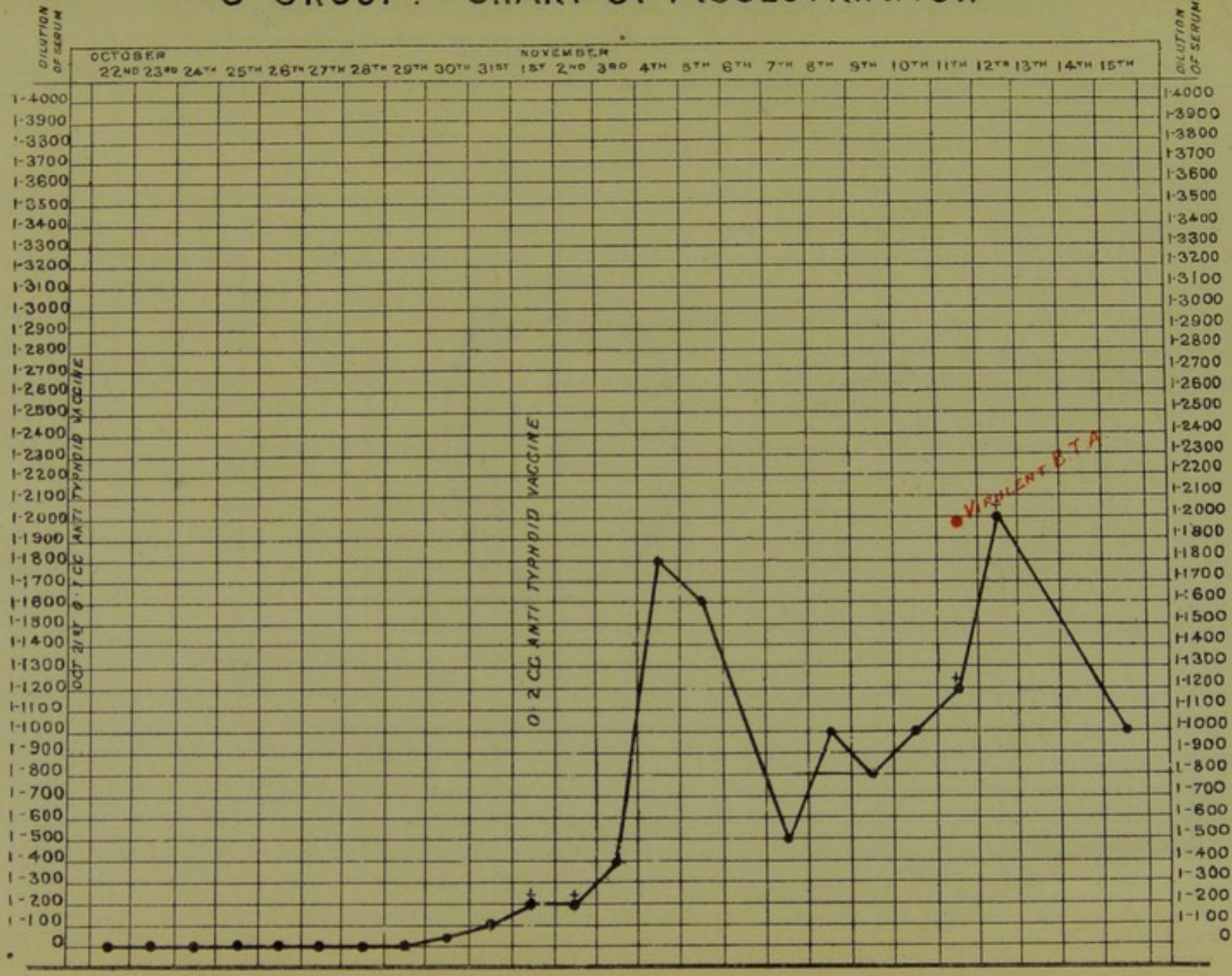
C GROUP:- CHART OF AGGLUTINATION

CHART 3



+ = HIGHEST DILUTION OF THE SERUM TRIED

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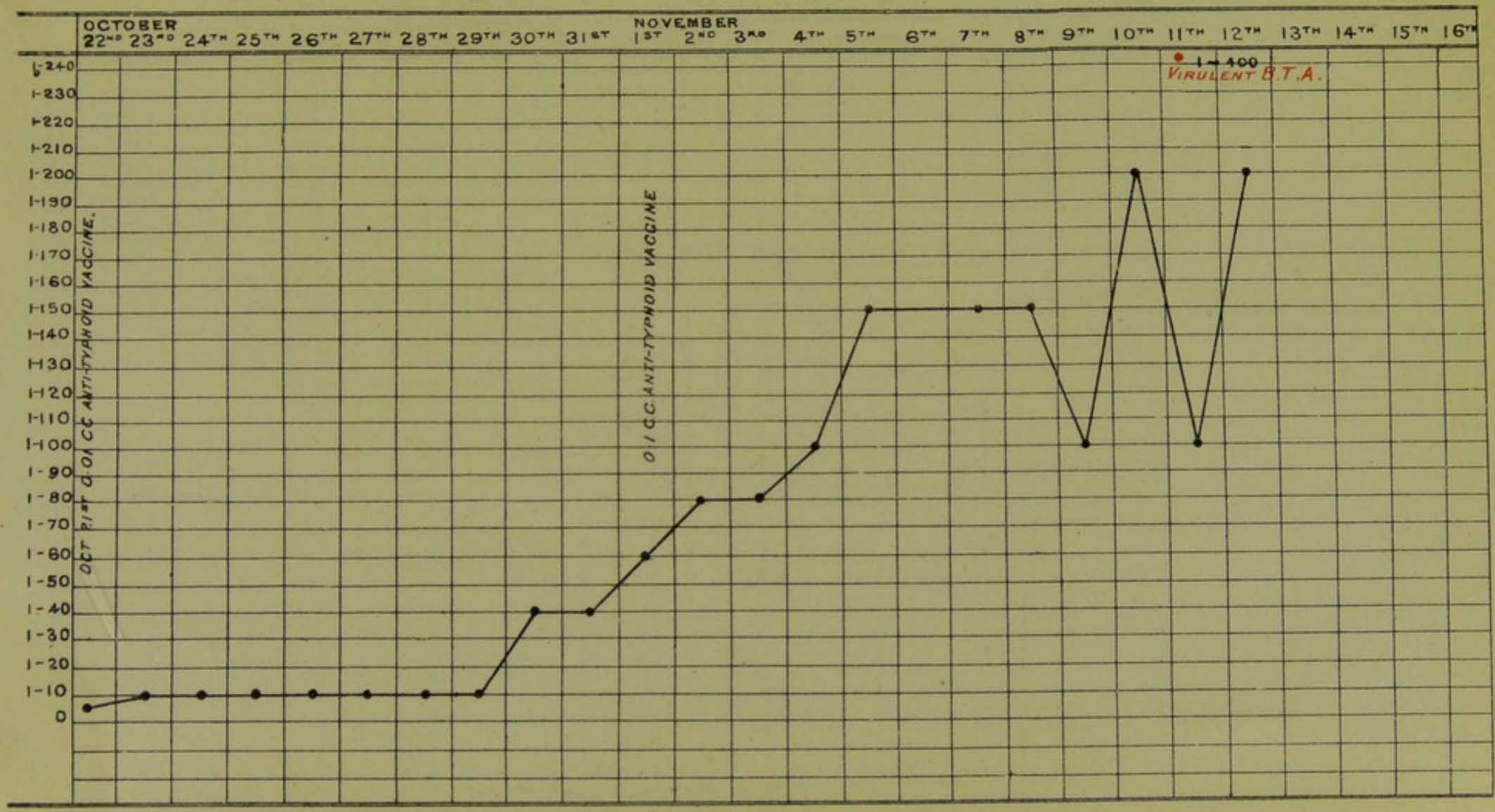
D GROUP

CHART OF AGGLUTINATION (ON ENLARGED SCALE)

CHART 4



+ = HIGHEST DILUTION TRIED

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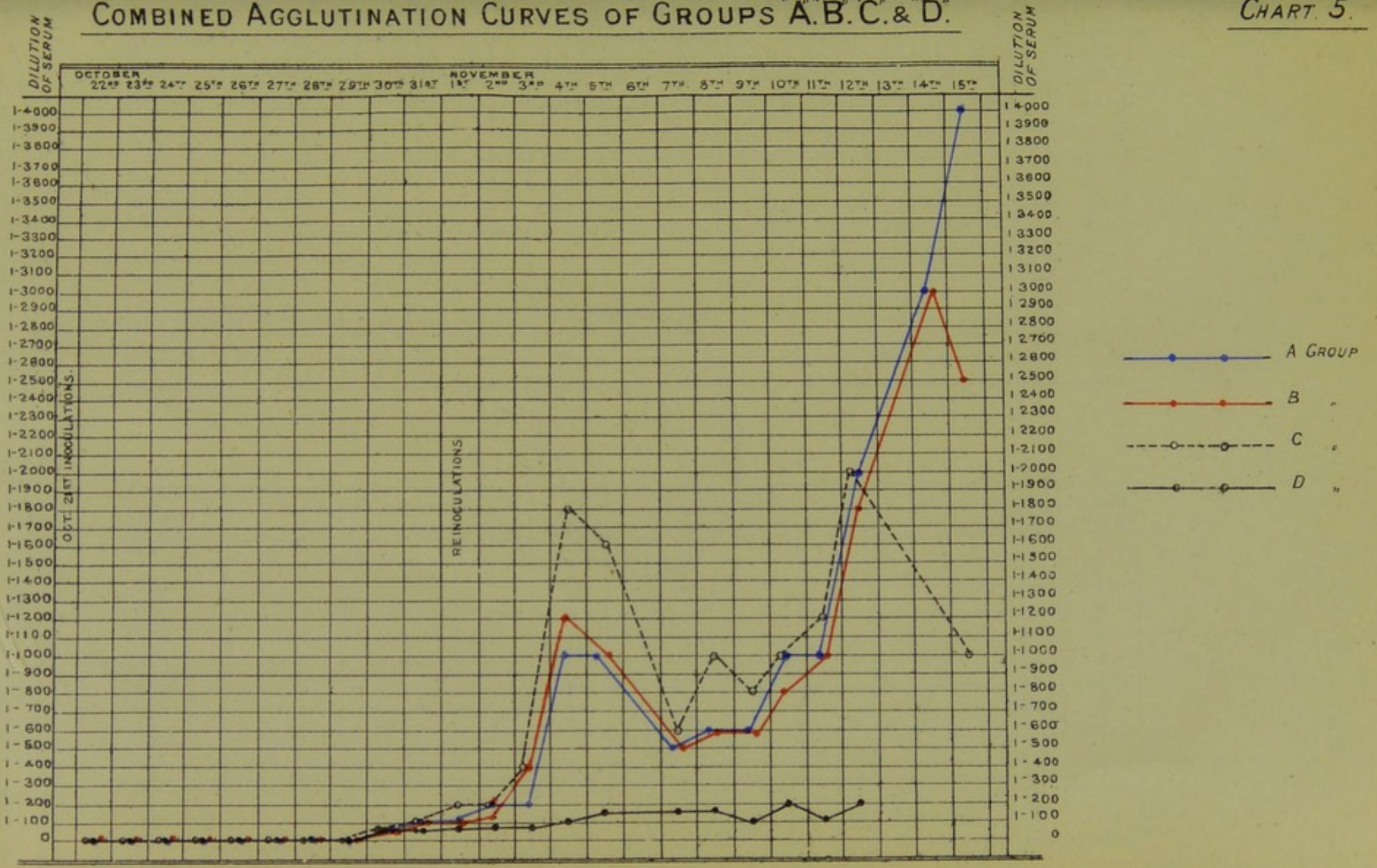
Combined Agglutination Curves of Groups "A." "B." "C." & "D."

Chart. 5.



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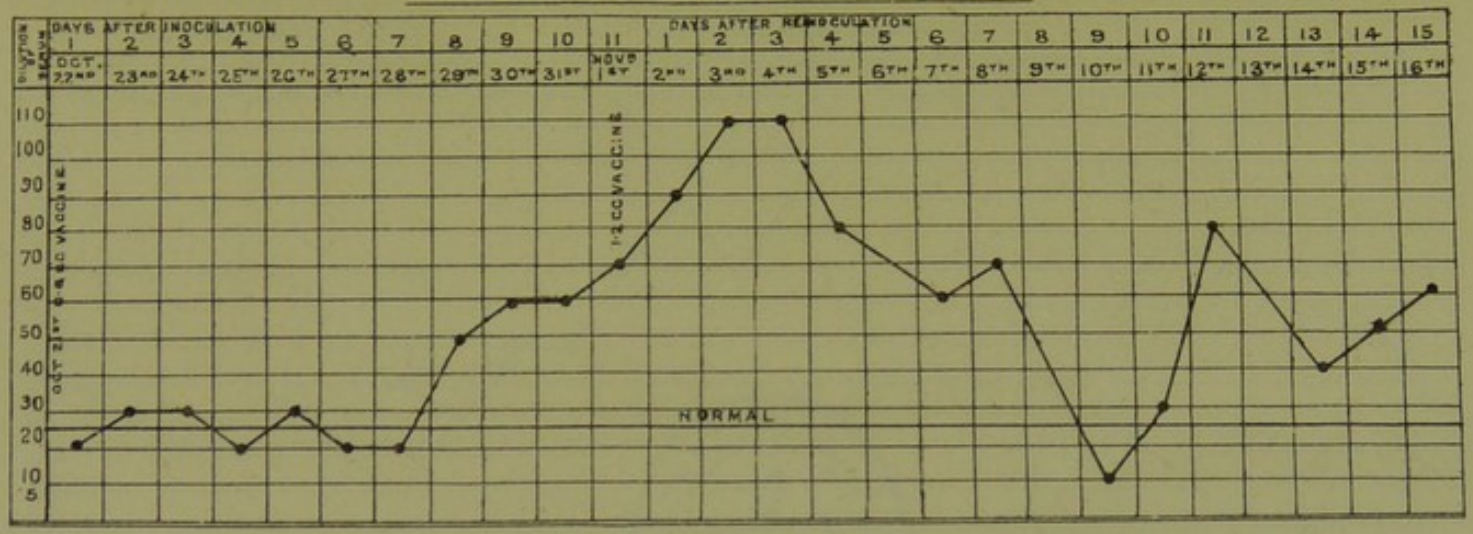
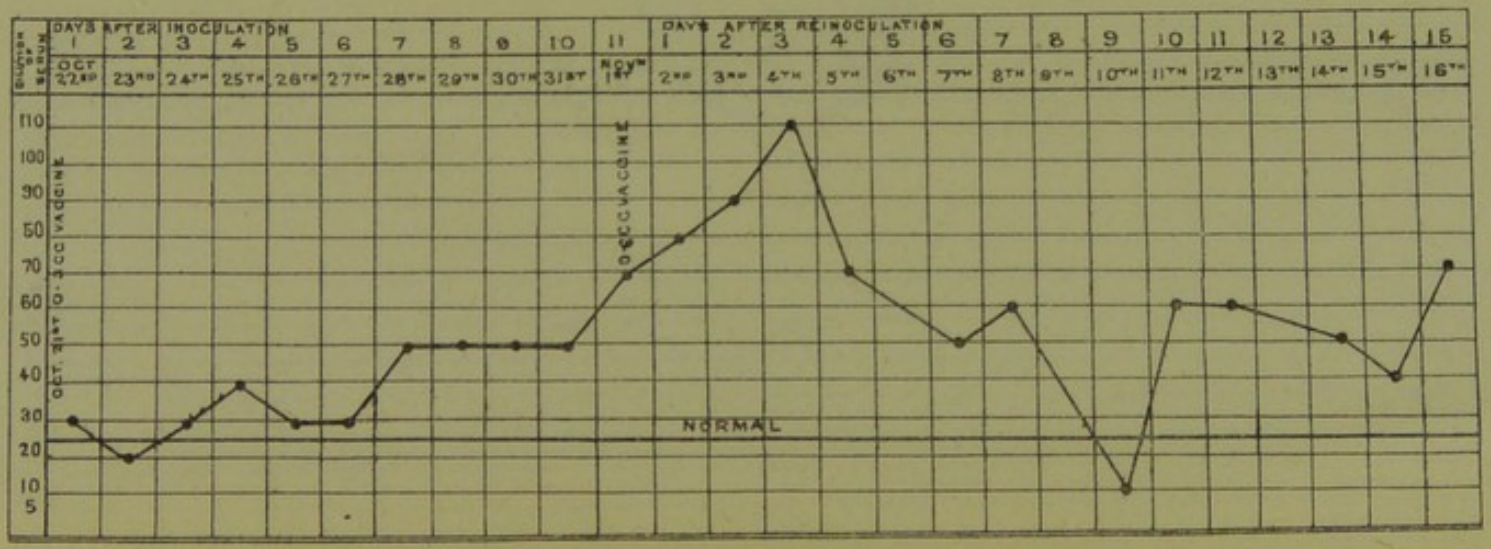
"BACTERICIDAL SUBSTANCES."

CHART 6

A GROUP



B GROUP



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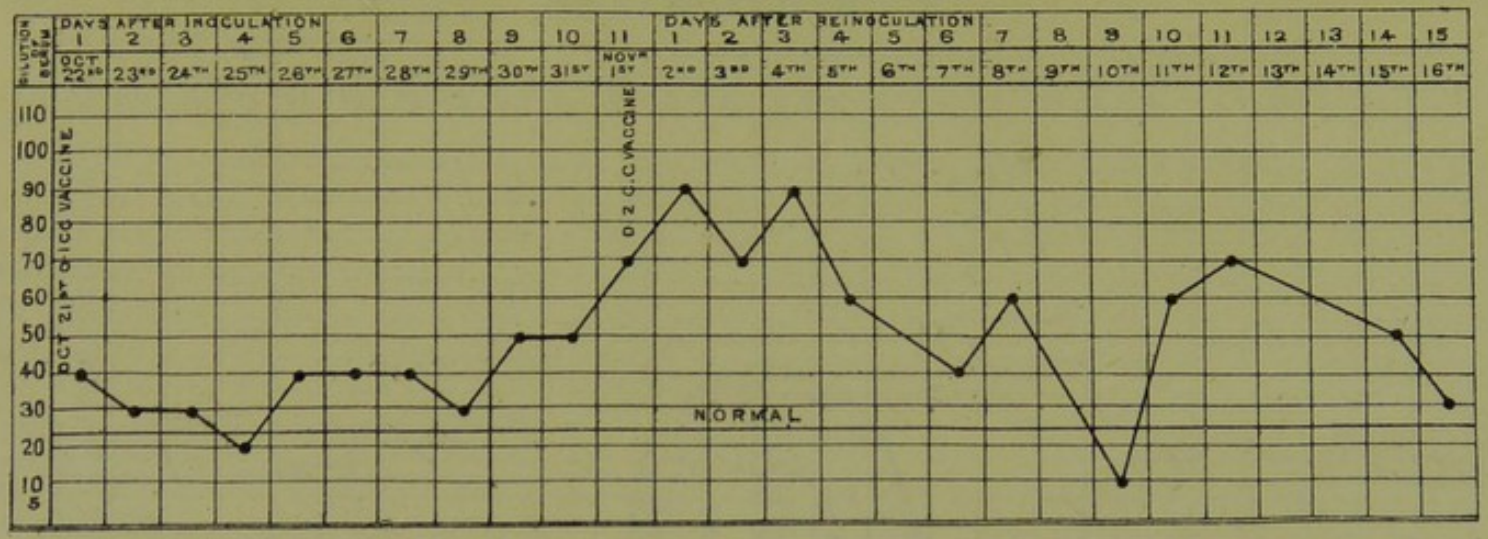
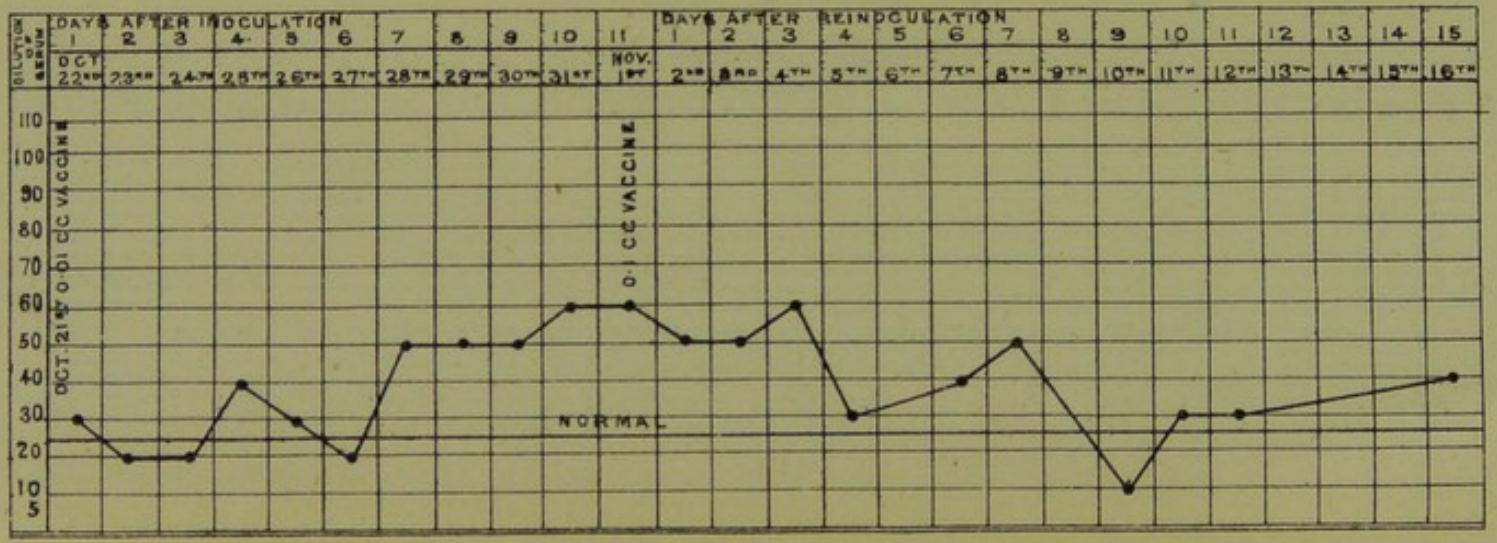
C Group

"Bactericidal Substances."

Chart 6
(Continued.)



D Group



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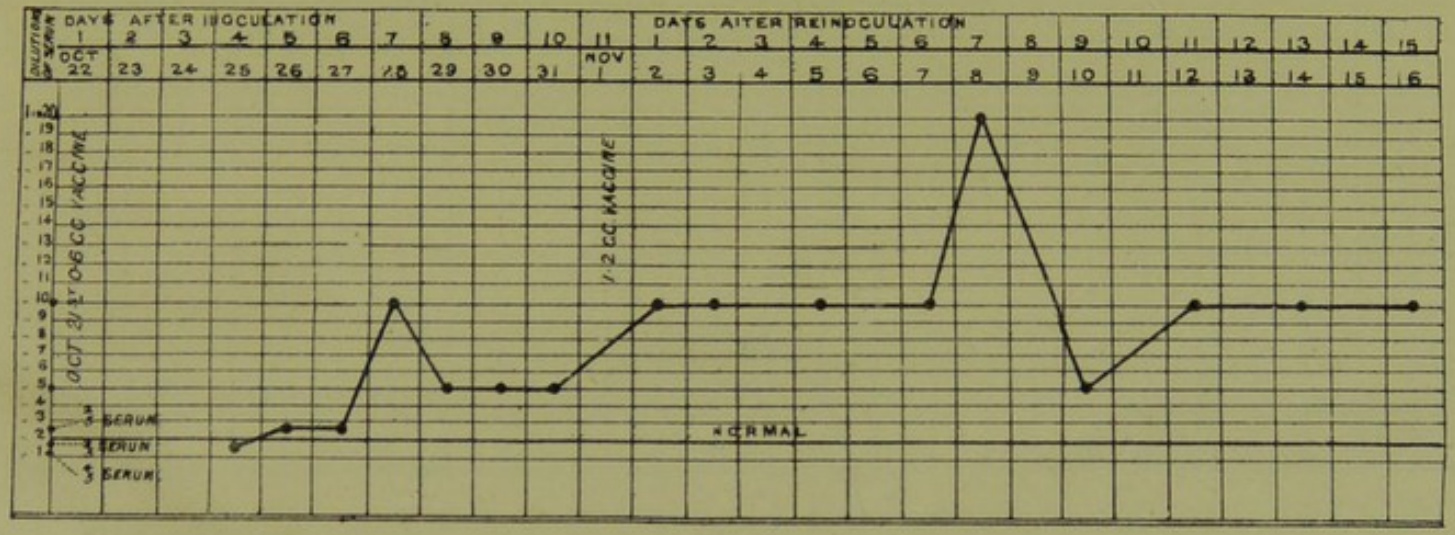
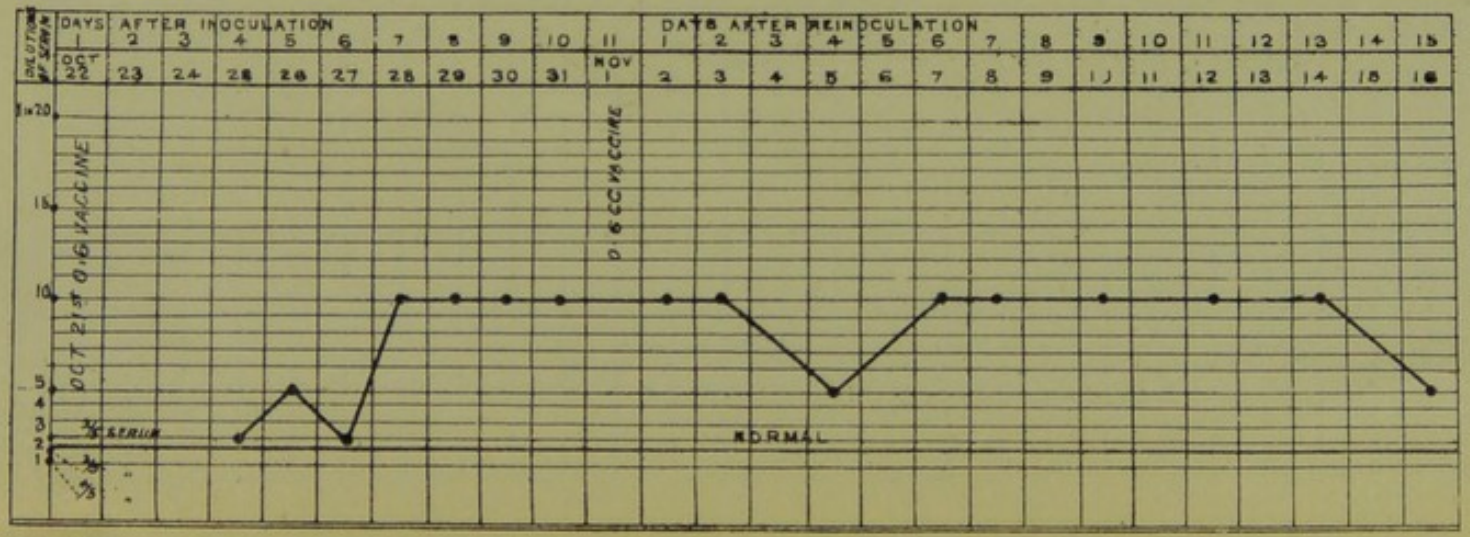
GROUP "A"

"BACTERIOLYSINS"

CHART 7



GROUP "B"



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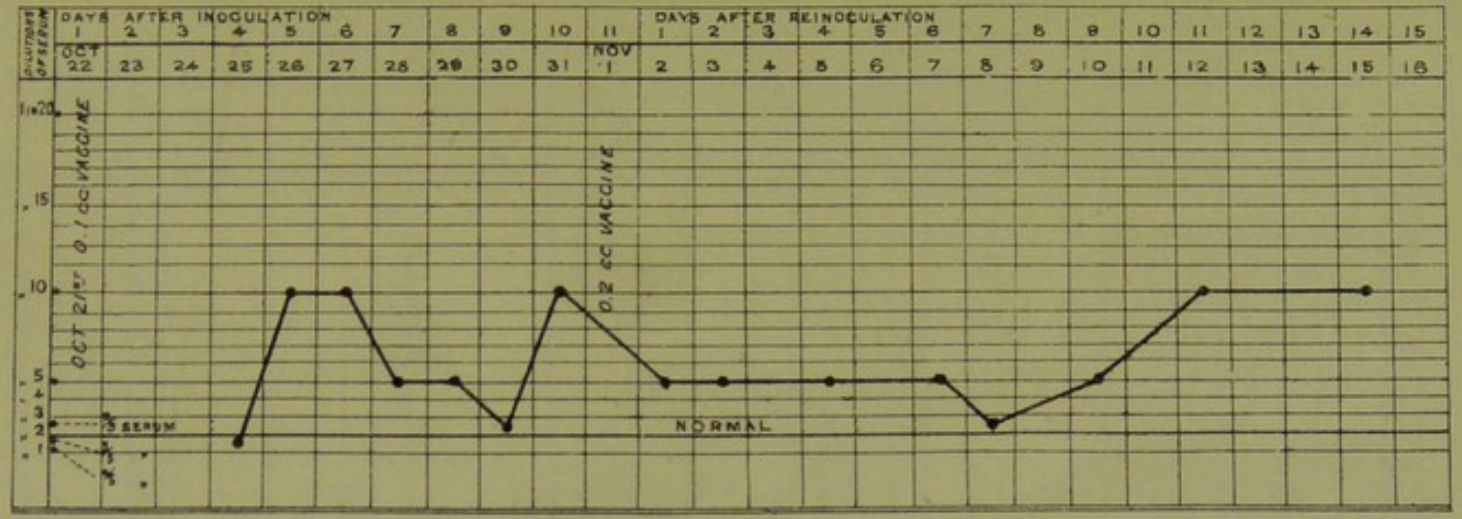
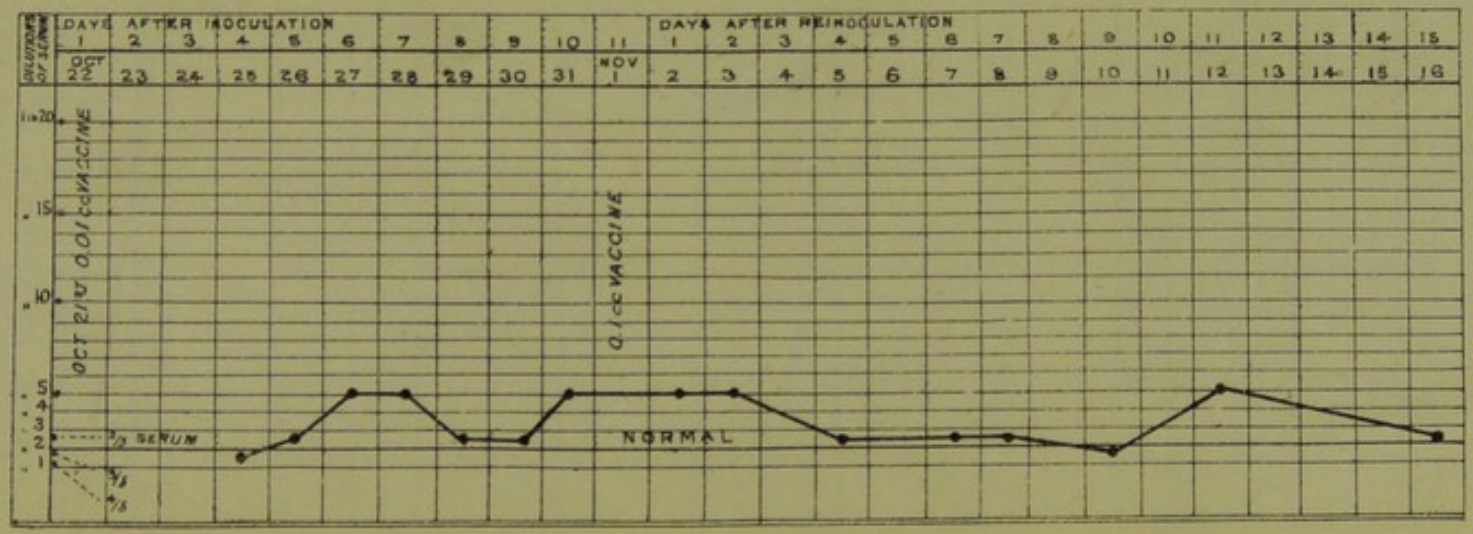
GROUP "C"

"BACTERIOLYSINS."

CHART 7. (CONTINUED)



GROUP "D"



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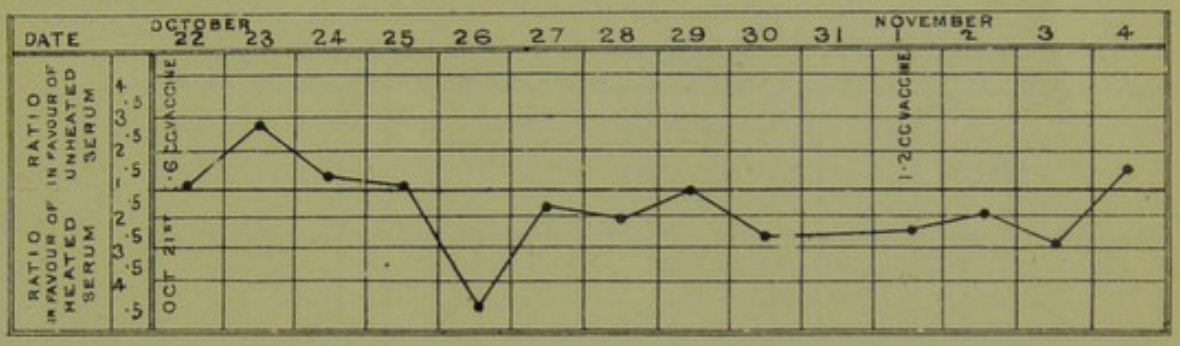
"OPSONINS"

CHART 8

GROUP "A"



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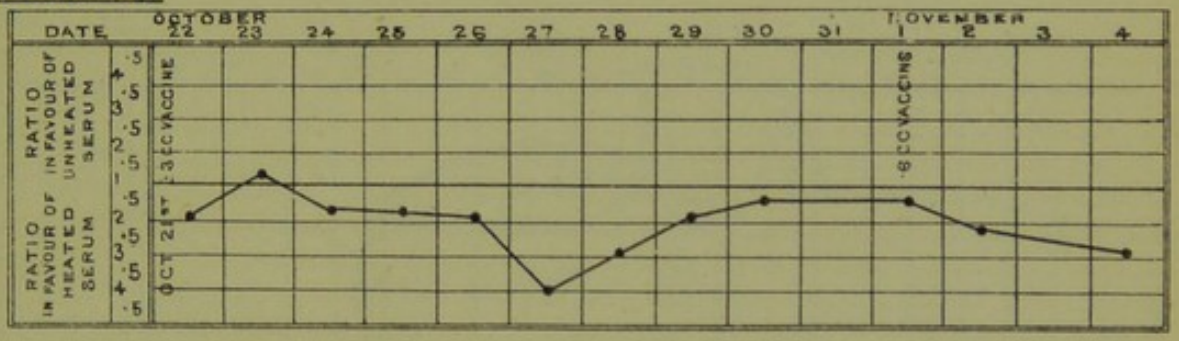

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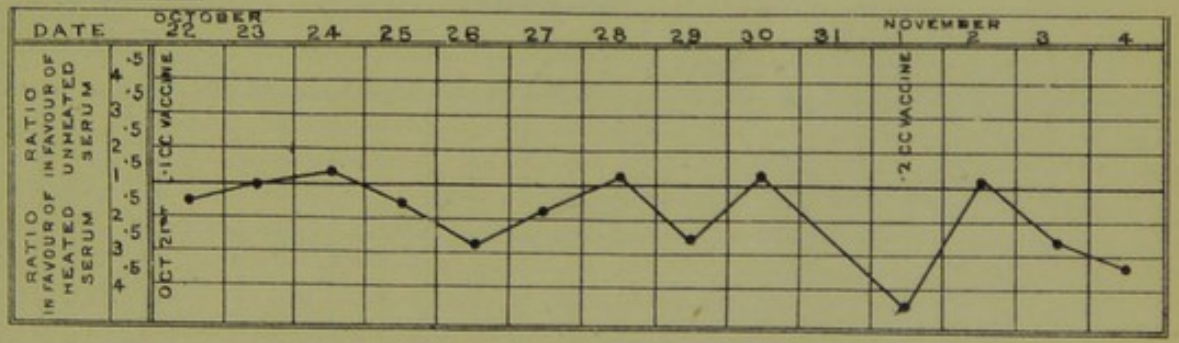

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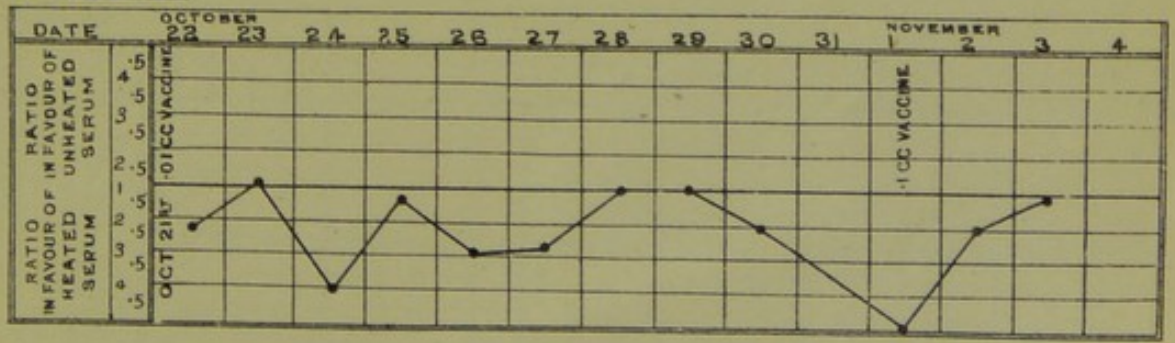

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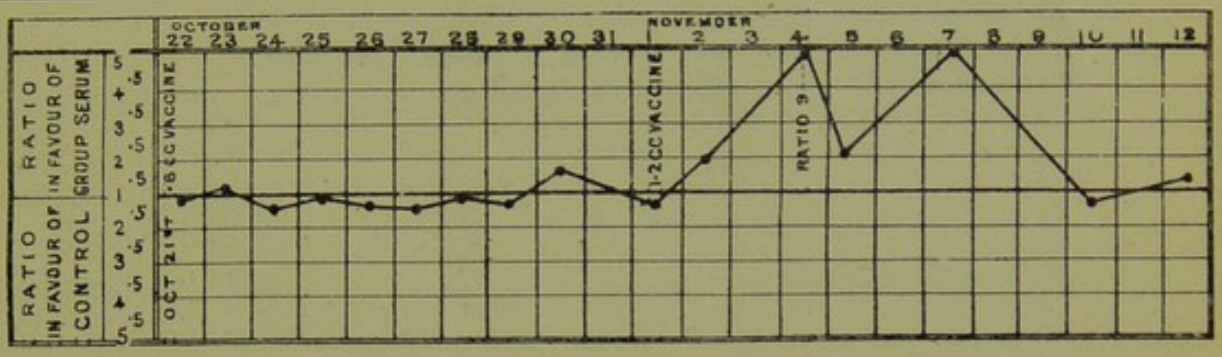
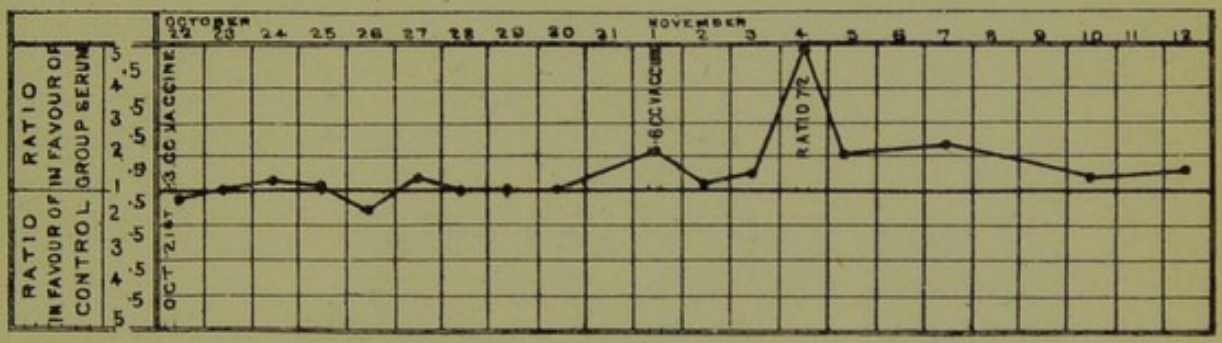
"STIMULINS"

CHART. 9.

GROUP "A"



GROUP "B"



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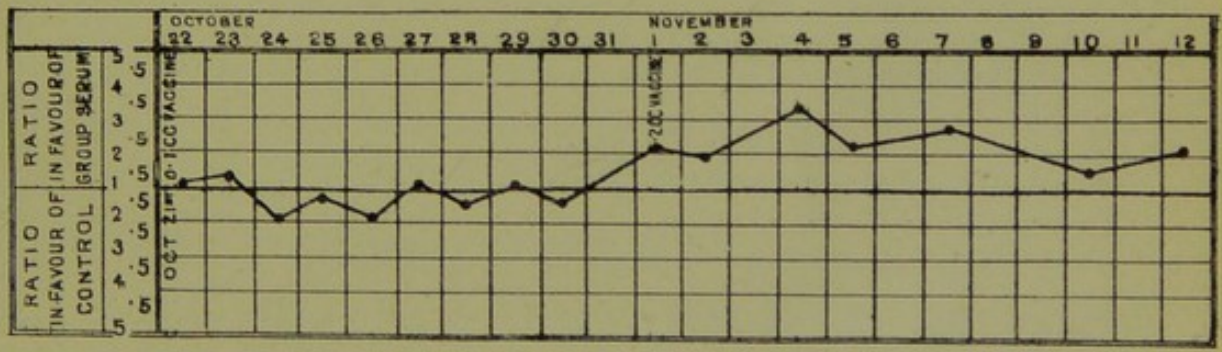

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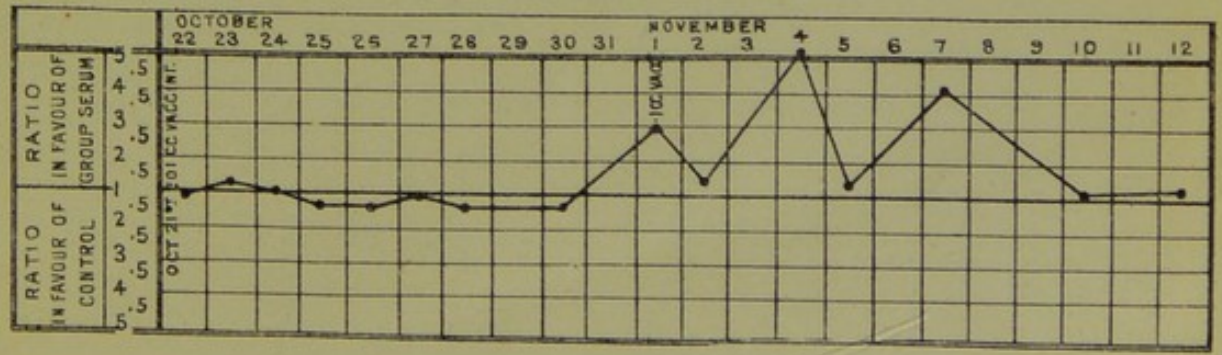

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