REPORT ON THE RESULTS OF EXPERIMENTS IN CONNECTION WITH ANTI-TYPHOID VACCINE.

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Experiments on Various Methods of Killing the Cultures.

Desiccation.—Emulsions of *Bacillus typhosus*, of three different strains, were made in sterile distilled water and placed in a vacuum exsiccator over sulphuric acid at 37° C. with the following results :—

First Experiment.—Emulsions sterile in twenty-four hours.

Second Experiment.—Emulsions sterile in seventy-two hours.

These experiments were made with small quantities of emulsion in watch glasses; when, however, larger quantities of emulsion were used complete sterilisation was not obtained, and it was found that a limit was placed to the length of time during which the specimens could be exposed in the exsiccator by the fact that, after six days, the resulting substance formed a tough membranous material which it was impossible to emulsify; this change took place quite quickly between the fifth and sixth days.

Third Experiment.—Five cc. of an emulsion of *B. typhosus* in water were placed in each of three watch glasses and one lot of 2 cc. was placed in a fourth glass. The samples were placed in the exsiccator with the result that the glass which had contained 2 cc. of emulsion was sterile at the end of two days, while the glasses which had contained 5 cc. were not sterile at the end of six days, by which time it was impossible to emulsify them, though, at the end of five days, they had emulsified quite readily. This phenomenon has occurred every time an emulsion has been dried for more than five days.

Fourth and Fifth Experiments.—A very thick emulsion of *B. typhosus* in 1 cc. of water gave a few isolated colonies only, when planted on to agar, at the end of four days' desiccation.

It was thought that probably the bacteria which survived owed their escape to being buried in the depths of the scales formed by drying the emulsion, where they would escape complete desiccation. Several attempts were made to overcome the difficulty by increasing the area over which the emulsion was spread and by aerating the emulsion before placing it in the exsiccator, so that when the

vacuum was produced the escaping air blew the emulsion into a fine froth; these expedients were, however, only partially successful, and it was considered that this method of desiccation could not be relied on to produce a sterile vaccine, except by way of filtering the emulsions of desiccated bacteria after autolysis had occurred. It was found that the desiccated bacteria, so long as they had not been dried for more than five days, broke up on the addition of normal saline solution into an emulsion which frothed easily and which, at the end of three days in the incubator at 37° C., was found, on microscopic examination, to have become almost completely autolysed, only an occasional unaltered rod being visible in stained specimens, which consisted of masses of granular *débris*. On filtering the autolysed emulsion an opalescent, slightly yellow fluid was obtained, which gave a flocculent precipitate with alcohol on heating to boiling point and on the addition of solutions of salts of the heavy metals, but no precipitate on the addition of nitric acid. It is proposed to take up the further study of the products of the autolysis of desiccated typhoid bacilli along with that of the products of autolysis got in other ways.

Chloroform.—Emulsions of the bacteria in normal saline, or cultures in broth, were put into an apparatus connected with a water pump, and so arranged that a stream of air was drawn first through a bottle containing chloroform and then through the culture or emulsion. At the end of the experiment the chloroform bottle was disconnected and air only was drawn through the culture for an hour, so as to free it from chloroform. A number of experiments of this kind were done, and it was found, in all cases, that an exposure for one hour to the chloroform-saturated air was sufficient to kill the bacteria. It was noticed that emulsions killed in this way frothed easily on shaking just as did emulsions of desiccated bacteria, and examination of stained specimens showed that autolysis occurred, but that it did not take place so rapidly as in emulsions of desiccated bacteria; as a rule, exposure of the chloroformed cultures for ten days at 37° C. was necessary before autolysis was complete; at the end of that time practically all recognisable bacteria were absent from stained specimens, which showed just a mass of formless, ill-staining granules.

Heat.—The emulsions or cultures, as the case might be, were sealed up in glass capsules and completely immersed in a water-bath at the required temperature. The following were the results:—

50° to 51° C. for 72 hours = not sterile.
52° C. for 1 hour = diffuse growth when planted on agar.
52° C. for 2¼ hours = gave growth of isolated colonies on agar.
52° C. for 3 hours = gave a single colony on agar.
52° C. for 24 hours = sterile.
53° C. for ½ hour = not sterile.
53° C. for 1 hour = sterile.
55° C. for ¼ hour = sterile.

From these results it appeared that, for an exposure of one hour, 53° C. is the minimum temperature at which one can ensure sterility of a culture of *B. typhosus*. The experiment has been repeated many times and with three different strains of the bacterium; the result has been the same in all cases. It was found, however, when working with larger quantities of material, 2 litres and upwards, that it was necessary to take very special precautions to ensure that the heat reached every portion of the fluid, otherwise a few stragglers survived. It was also found necessary to check the readings of one's laboratory thermometers by means of a standard instrument, since most of those in general use will be found to give an error of a degree, or even more, at some portion of the scale.

Autolysis was found to occur in cultures killed by an exposure to 53° C. for one hour, but it did not occur nearly so rapidly, nor was it as complete, as in the case of either the chloroformed cultures or of emulsions of desiccated bacteria. Several observations were made, *apropos* of this point, in cultures heated to 55° C. and 60° C., and it was found that in these cases there was practically no autolysis recognisable under the microscope, whereas in cultures killed by heating to 52° C. for twenty-four hours marked autolysis was noticed after two days' incubation at 37° C.

Alcohol.—Varying proportions of absolute alcohol were added to a broth culture of *B. typhosus*. In the first experiment it was found that 10 per cent. alcohol just failed to kill all the bacteria in two days at 37° C., while 20 per cent. alcohol produced complete sterility in this time. In a second experiment, using an emulsion of the micro-organisms in saline, 10 per cent. alcohol produced sterility in twenty-four hours, so that the proportion of alcohol necessary to kill *B. typhosus* in twenty-four hours seems to be somewhere about 10 per cent. Examination of emulsions killed by this quantity of alcohol in twenty-four hours, gave evidence of no trace of autolysis having occurred after seven days' incubation of the killed cultures at 37° C.

Toluol.—The use of this substance was suggested by the fact

that it is largely employed for the preservation of anti-sera and toxins from contamination, and in experiments on the autolysis of animal tissues. In the first experiment a layer of toluol, about $\frac{1}{4}$ inch deep, was run on to the surface of a twenty-four hour broth culture of *B. typhosus* in a tube, the tube was capped with rubber and placed in the incubator at 37° C.; after twenty-four hours' exposure a culture on agar gave a single colony, and on the third day the culture was found to be sterile. The rubber cap was then removed and the toluol allowed to evaporate, which it did completely at the end of two days. On the fifth day after the addition of toluol marked autolysis was found to have occurred in the culture, very few unaltered rods being present; many of the bacteria were spherulated, and the majority of those forms which could be recognised as the remains of bacteria just showed the "ghosts" of outlines of rods or spheres.

In a second experiment, with an emulsion of *B. typhosus* in saline, sterilisation was complete in twenty-four hours and autolysis practically complete at the end of seven days. From these and other experiments it would appear that one can rely on toluol to kill a culture or emulsion of typhoid bacilli within three days.

Experiments with different strains of the bacterium show that autolysis occurs much more rapidly with some strains than with others, and that it is more rapid in emulsions made with distilled water than in the case of those made with saline solution; this last is, however, only a temporary advantage, for the ultimate result as regards autolysis appears to be practically the same whether distilled water or saline be used for the making of the emulsions.

Some experiments were made with a view to determining whether the addition of alkalies or acids in small quantity would further the process of autolysis; the results showed that a very small quantity of alkali, or the addition of sufficient acid to produce an acid reaction in the emulsions, completely inhibited autolysis. From this, as well as from the effects of heating beyond 53° C., it would appear that the process of autolysis is something of a chemical nature, probably depending on a ferment. The point is of interest, since several writers (Neisser and Shiga, Wasserman and others) describe the preparation of a filtrate vaccine by "autolysis" of a culture which has been heated to 60° C. for one hour; it would seem that the fluid which they obtain in this way is rather the result of simple diffusion of the contents of the dead bacilli into the surrounding fluid than of a true autolysis.

Filtrates of emulsions killed by toluol and afterwards submitted

to autolysis, gave similar reactions to those given by filtrates of autolysed desiccated bacteria. It was found that toluol kept in the ordinary way in bottles could not be relied on to be free from spore-

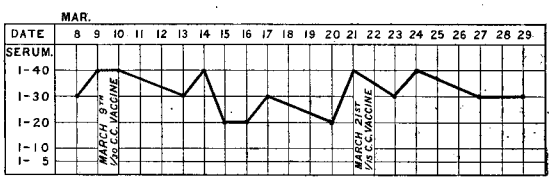

CHART I.—Bactericidal action of the serum of a rabbit which received $\frac{1}{30}$ cc. of a vaccine (killed at 65° C.) on March 9, and $\frac{1}{15}$ cc. of the same vaccine on March 21.

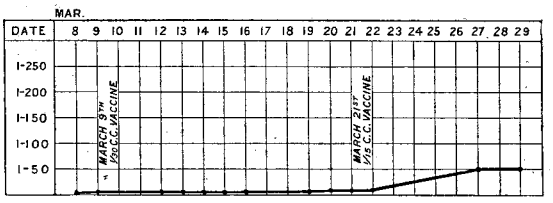

CHART II.—Agglutination value of the serum of a rabbit which received $\frac{1}{30}$ cc. of a vaccine (killed at 65° C.) on March 9, and $\frac{1}{15}$ cc. of the same vaccine on March 21.

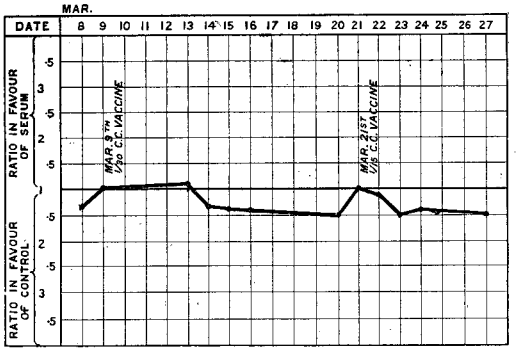

CHART III.—"Stimulin" action of the serum of a rabbit which received $\frac{1}{30}$ cc. of a vaccine (killed at 65° C.) on March 9, and $\frac{1}{15}$ cc. of the same vaccine on March 21.

bearing organisms, but it was easily sterilised by boiling for half an hour in sealed capsules, the test organism being *B. mesentericus*.

Glycerine.—Emulsions were made in varying strengths of glycerine and then placed in the incubator at 37° C., with the following results :—

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

Experiments on the Anti-tropic Substances Produced by Vaccines Prepared in Various Ways.

The technique employed for the measurement of these anti-tropic substances was the same as that employed at Aldershot during investigation into the blood changes consequent on anti-typhoid inoculation in man.[1]

First Experiment.—This was done in order to ascertain what effect overheating the culture would have on its value as a vaccine. An emulsion in saline of a twenty-four hour agar culture of *B. typhosus* (R), giving a count of 1,283 millions per cc., was heated to 65° C. for twenty minutes, and injected into a rabbit in doses of $\frac{1}{30}$ cc. and $\frac{1}{15}$ cc., with an interval between the doses of eleven days. The results of the experiment are shown in the accompanying charts (I. to III.). From these it will be seen that, after injection of a culture heated to 65° C., there was no rise in the bactericidal power of the rabbit's serum, very late and insignificant production of agglutinins and no evidence of the formation of stimulins. It would appear, then, that to overheat a culture of *B. typhosus* seriously impairs its vaccinating properties. These results agree with those of Friedberger and Moreschi,[2] who found that the higher the temperature at which a culture was killed the less its vaccinating properties.

Second Experiment.—The object of this experiment was to find out whether an emulsion of *B. typhosus* which had been killed by chloroform possessed any value as a vaccine. An emulsion of a twenty-four hour culture of typhoid bacilli was made in saline and killed by chloroform; a count of the emulsion showed 2313·95 million bacteria per cubic centimetre, or about twice as many as in the experiment quoted above. A rabbit was given $\frac{1}{60}$ cc. of the killed culture, and, eleven days later, another dose of $\frac{1}{30}$ cc. The results of the examination of the serum are to be seen in the accompanying charts (IV. to VI.). It will be seen that there was a rise in the bactericidal power of the rabbit's serum, and that the

[1] Leishman, Royal Army Medical Corps Journal, July, 1905.

[2] Friedberger and Moreschi, *Cent. für Bakt.*, xxxix., September 22nd, 1905, pp. 453-473.

chart follows very much the same lines as in vaccinated man. The same remarks apply also to the agglutinins, with the exception that they seem to have appeared rather earlier than they do in man.

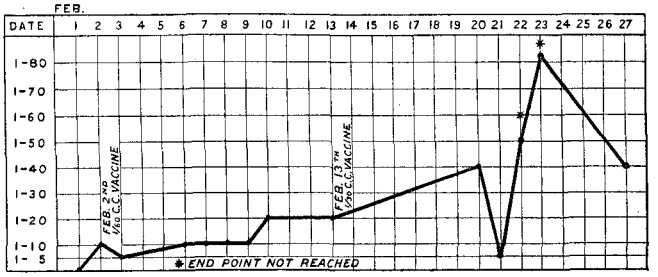

CHART IV.—Bactericidal action of the serum of a rabbit which received $\frac{1}{60}$ cc. of a vaccine (killed by chloroform) on February 2, and $\frac{1}{30}$ cc. of the same vaccine on February 13.

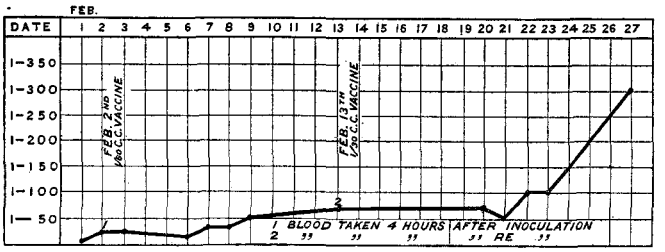

CHART V.—Agglutination value of the serum of a rabbit which received $\frac{1}{60}$ cc. of a vaccine (killed by chloroform) on February 2, and $\frac{1}{30}$ cc. of the same vaccine on February 13.

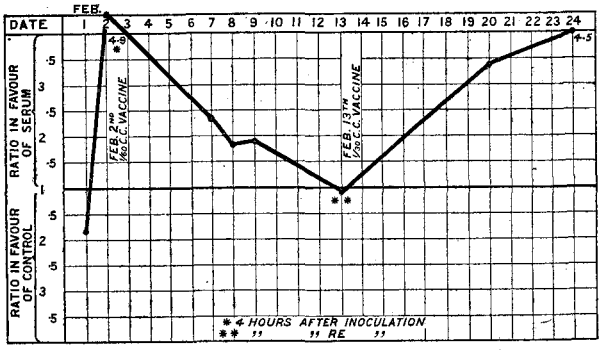

CHART VI.—"Stimulin" action of the serum of a rabbit which received $\frac{1}{60}$ cc. of a vaccine (killed by chloroform) on February 2, and $\frac{1}{30}$ cc. of the same vaccine on February 13.

With regard to "stimulins," there was a most remarkable and sudden appearance of a stimulin effect from serum taken only four hours after the first dose; this phenomenon was not repeated after

the second (and larger) dose, which was followed by a temporary drop in the stimulin value of the serum. It would appear from the results of subsequent experiments that the high stimulin value produced in this particular rabbit's serum was due to an individual peculiarity in the rabbit, for, though evidence of the formation of stimulins was found in the experiment next to be recorded, they never reached so high a level as in the present case.

Third Experiment.—This experiment was made for the purpose of making a comparative study of the anti-tropic substances produced by the injection into rabbits of vaccines of precisely the same strength but which had been killed in different ways. An emulsion of *B. typhosus* in saline, of a strength of 1,283 million bacteria per cubic centimetre, was made and divided into three portions; one portion was killed by heat at 60° C., one by heating to 53° C., and one by chloroform. Doses of $\frac{1}{30}$ cc. of vaccine were given to, in each case, three rabbits; fourteen days later doses of $\frac{1}{15}$ cc., and fourteen days later again doses of $\frac{1}{2}$ cc. were similarly given. The object of giving the large dose at the end was with a view to accentuating any differences there might be in the results of the inoculation of the different vaccines. The observations were made on the pooled sera of the rabbits of each group, so as to eliminate as far as possible the variations due to individual peculiarities in the animals. From the charts (VII. to IX.) it will be seen that the bactericidal curves followed approximately a parallel course in all cases, whereas in the case of agglutinins the rabbits vaccinated with the chloroformed culture gave a much feebler reaction. The stimulin curves show that, in this particular, the chloroformed vaccine had the advantage over the vaccine killed at 53° C., and that in the case of the vaccine killed by heating to 60° C., there was no stimulin effect produced from the doses of vaccine given.

An experiment was made to see if a comparison of the phagocytic index of the sera of the different groups gave any confirmation of the findings with regard to stimulins. The results were as follows:—

(1) Normal rabbit corpuscles, 3 vols. Heated normal rabbit serum, 3 vols. Emulsion of *B. typhosus*, 1 vol.	15″ at 37° C.	Average bacteria per phagocyte, 2·2.
(2) Normal rabbit corpuscles, 3 vols. Heated serum of vaccinated rabbits (60° C., vaccine), 3 vols. Emulsion of *B. typhosus*, 1 vol.	15″ at 37° C.	Average bacteria per phagocyte, 3·1.

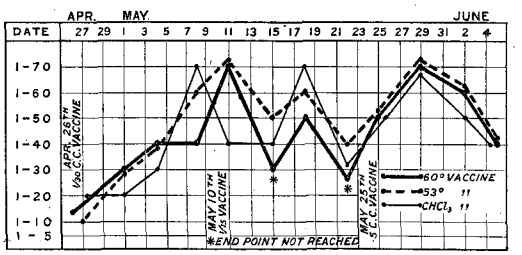

CHART VII.—Bactericidal action of sera of three groups of rabbits, which received doses of anti-typhoid vaccine, killed (1) at 60° C.; (2) at 53° C.; (3) by chloroform. First dose, $\frac{1}{30}$ cc. on April 26; 2nd dose, $\frac{1}{15}$ cc. on May 10; 3rd dose, $\frac{1}{2}$ cc. on May 25.

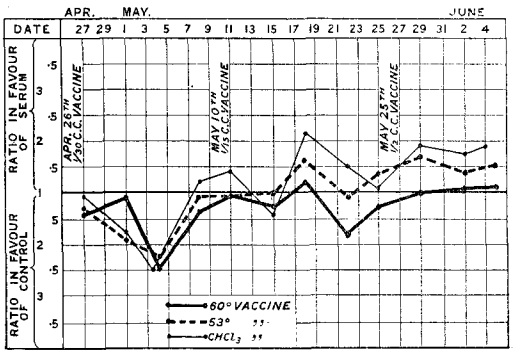

CHART VIII.—"Stimulin" action of the sera of three groups of rabbits, which received doses of anti-typhoid vaccine, killed (1) at 60° C.; (2) at 53° C.; (3) by chloroform. First dose, $\frac{1}{30}$ cc. on April 26; 2nd dose, $\frac{1}{15}$ cc. on May 10; 3rd dose, $\frac{1}{2}$ cc. on May 25.

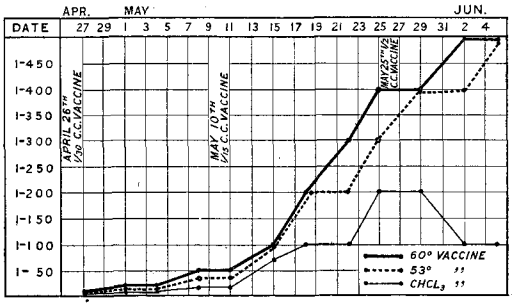

CHART IX.—Combined agglutination chart of the sera of three groups of rabbits which received doses of vaccine, killed by heat at 60° C. and at 53° C., and by chloroform. First dose, $\frac{1}{30}$ cc. on April 26; 2nd dose, $\frac{1}{15}$ cc. on May 10; 3rd dose, $\frac{1}{2}$ cc. on May 26.

(3) Normal rabbit corpuscles, 3 vols. Heated serum of vaccinated rabbits (53° C., vaccine), 3 vols. Emulsion of *B. typhosus*, 1 vol.	15″ at 37° C.	Average bacteria per phagocyte, 5.
(4) Normal rabbit corpuscles, 3 vols. Heated serum of vaccinated rabbits ($CHCl_3$ vaccine), 3 vols. Emulsion of *B. typhosus*, 1 vol.	15″ at 37° C.	Average bacteria per phagocyte, 4·4.

Control Experiments as to the normal bactericidal power of rabbit serum (six experiments) gave the average dilution, which was effective on a 1—10,000 dilution of a twenty-four hour broth culture of typhoid bacteria, as 1—20, the lowest being 1—5 (twice) and the highest 1—40 (once). The effect of the addition of one volume of a 1—5 diluted rabbit serum to a mixture of human serum, human corpuscles and emulsion of typhoid bacteria was, in every case, to lower the phagocytic index slightly, but the effect was no more than could quite well be explained by the dilution effected in the human serum by the addition.

Fourth Experiment.—This was done to see if the filtrate from an autolysed culture of typhoid bacilli, which had been killed by toluol, had any value as a vaccine. An emulsion was made by adding 1 cc. saline to each of several twenty-four hour agar growths of a virulent culture of *B. typhosus;* after being killed by toluol, it was allowed to autolyse and was then filtered through a Kitasato candle. The resulting fluid, which was of a clear, bright yellow appearance, was collected in glass capsules and hermetically sealed; after two days it was found to be slightly turbid and contamination was suspected, more especially when, later on, flocculi appeared in the tube; cultures were made from the filtrate, both anaerobically and aerobically, and they proved to be sterile, while microscopic examination of the fluid gave no evidence of the presence of micro-organisms, either bacteria or yeasts, both of which were suspected. It was evident, therefore, that the turbidity was due to a precipitation of some of the probably albuminous material contained in the fluid; what the exact nature of the process is and what influence, if any, it has on the vaccinating properties of the filtrate has, so far, not been ascertained.

Two well-grown rabbits were given each 1 cc. of the fluid hypodermically on September 11th, and a further dose of 2 cc. on October 5th. The inoculation was not followed by any apparent alteration in the health of the animals.

In the subsequent observations a modification was introduced

by comparing the bactericidal power of the grouped sera of the two vaccinated rabbits with the results of a simultaneous observation on the grouped sera of two normal rabbits; one is able, in this way, to construct a curve from which the variations due to accidental circumstances have been more or less obliterated, and thus to obtain a more just appreciation of the effect of the inoculation on the rise and fall of the bactericidal power of the serum

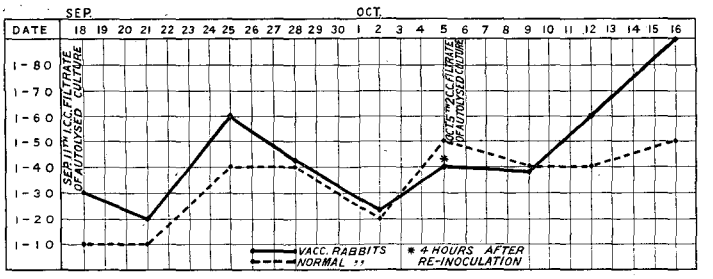

CHART X.—Comparative chart of the bactericidal action of the grouped sera of two rabbits, inoculated on September 11 and October 5 with the filtrate of an autolysed emulsion of *B. typhosus*, which had been killed by toluol, and of the grouped sera of two normal rabbits.

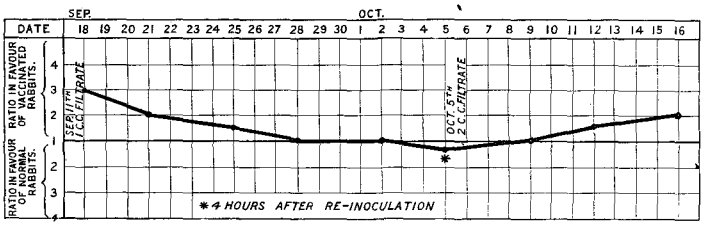

CHART XI.—Curve showing the rise and fall of the bactericidal action of the sera of two rabbits which had been inoculated with filtrate of an autolysed emulsion of *B. typhosus*, the bactericidal action of the grouped sera of two normal rabbits being treated as unity.

as compared with normal serum tested under the same conditions. It will be seen (chart No. XI.) that the sera of the vaccinated rabbits exercised its highest bactericidal properties on the seventh day (so far as was observed), and after that there was a steady drop to normal. After reinoculation there was some evidence of a fall below normal in the bactericidal action of the serum, and after that it rose, and was rising at the time the experiment was discontinued. It will be seen (charts X. to XIII.) that agglutinins were late in appearing and did not reach any great amount (in

this respect the vaccine resembled the chloroformed vaccines). There was a sudden drop in agglutinins as a result of reinoculation, and they subsequently rose again; but, so long as the experiment continued, they never rose to any great height. In the "stimulin" experiments, in order to obviate accidental differences in the readings, the experiments were done in duplicate, using different emul-

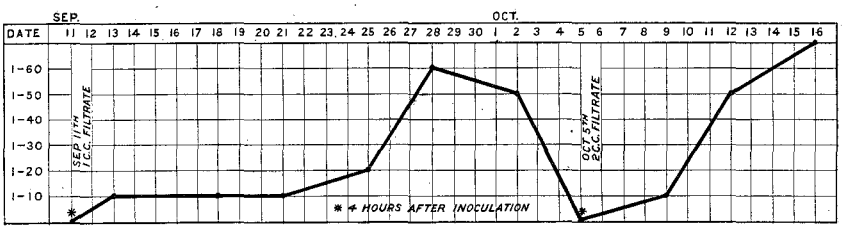

CHART XII.—Agglutination Chart (on an enlarged scale) of the grouped sera of two rabbits who were inoculated with filtrate of an autolysed culture of *B. typhosus* on September 11 and October 5.

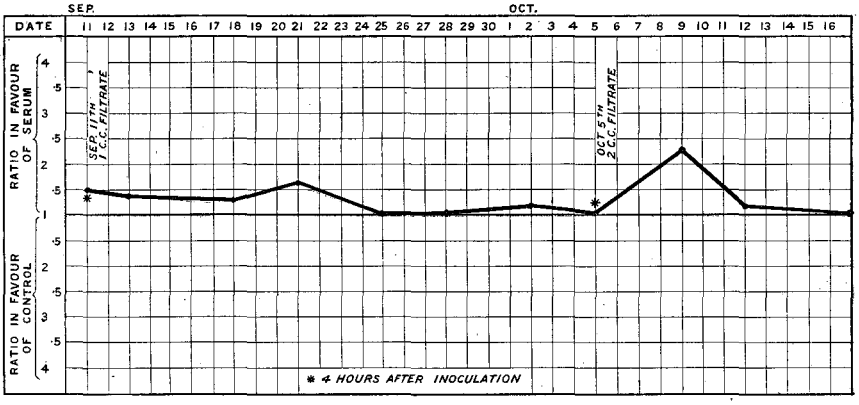

CHART XIII.—"Stimulin" action of the grouped sera of two rabbits which were inoculated with the filtrate of an autolysed culture of *B. typhosus* on September 11 and October 5.

sions, and the contents of not less than fifty cells were counted in each case, the result being stated as an average of the counts of the two experiments. The results in all cases were that the two experiments of each duplicate series confirmed each other. It will be seen that there is a sudden appearance of a stimulin action of the serum four hours after inoculation, just as occurred after the use of the chloroformed vaccine; that this stimulin action of the sera of the vaccinated rabbits lasted till the fourteenth day, when

it disappeared, and that reinoculation with a larger dose was not immediately followed by a reappearance of the substances which produce the stimulin phenomenon; when they did appear they lasted for only a short time and then disappeared altogether.

In another experiment made for the purpose of comparing the vaccinating properties of chloroformed cultures and those killed by toluol (unfiltered), the result was that whereas the injection of chloroformed culture caused the same rise of bactericidal power, &c., as in previous experiments with this substance, the serum of those rabbits which had been inoculated with toluol-killed cultures (unfiltered) showed a gradual and progressive decline in bactericidal power till, in the end, the serum showed no bactericidal power whatever. It is possible that this was due to extreme activity of the toluol-killed culture, which may have produced a prolonged "negative phase." Further experiments are necessary, however, before one can form a definite opinion on the subject.

Fifth Experiment.—The object of this experiment was to ascertain whether the swallowing of an emulsion of typhoid bacilli in glycerine would result in the formation of protective substances in the blood of man. The subject of the experiment (W. S. H.) had been inoculated a year previously with anti-typhoid vaccine, and his serum still gave evidence of this; the bactericidal power was effective in a dilution of 1—70 (normal 1—20) and agglutinins in a dilution of 1—200 (maximum); but the serum gave a phagocytic index no higher than normal, and showed no evidence of stimulins when added in small quantity to a mixture of normal serum, blood cells and bacteria.

An emulsion in sterile neutral glycerine was made from a mixture of virulent and avirulent growth of *B. typhosus* on agar, using 1 cc. for each agar slope. The emulsion was found to be sterile at the end of twenty-four hours, and as soon as sterility was confirmed doses commencing with 0·1 cc., and gradually increasing to 0·75 cc., were swallowed, daily at first and later every two or three days, until in all 4·5 cc. had been taken; the doses were swallowed as far as possible on an empty stomach.

The only symptoms that were noticed were very slight mental confusion, coming on about half an hour after the larger doses and lasting about an hour; this would not have been noticed had one not attempted at the time to do some arithmetical calculations. Samples of blood were taken fourteen days after the first dose and two hours after the last dose, and the serum was tested for the phagocytic ratio as compared with normal serum. It was found to

be + 2·4. This rise in the phagocytic ratio persisted for eight days and was accompanied by evidence of stimulins, as tested by Leishman's method; a week later it was found to have disappeared, and did not return although three further doses, amounting in all to 1·9 cc., were swallowed. There was a rise in the bactericidal power of the serum up to a maximum of 1—110 as compared with a normal of 1—10, this being found fifty-two days after the commencement of the treatment, and two months later the bactericidal power of the serum was active in a dilution of 1—80 as compared with a normal of 1—10. There was no rise in agglutinins, which remained at the point at which they were before treatment was commenced.

In order to see whether this was due to a destruction of the agglutinogens by the glycerine, a dose of 1 cc. of the glycerine emulsion was given to a guinea-pig hypodermically, and seventeen days later its serum was found to agglutinate typhoid bacilli in a dilution of 1—100; it is therefore probable that the absence of a rise in the agglutinins was due to the method of administration of the vaccine.

Sixth Experiment.—This was a repetition of the last experiment, this time on a normal man (T. H. G.) His blood had served as a "normal control" on several occasions, and was found to be bactericidal for typhoid bacilli in a dilution of 1—10; it contained no agglutinins for *B. typhosus*, it gave a normal phagocytic ratio and showed no evidence of the presence of stimulins.

An emulsion in glycerine of typhoid bacilli prepared as in the previous experiment, was administered by the mouth in gradually increasing doses, commencing with a small dose of 0·05 cc. (= 0·05 agar growth) and increasing to a maximum of 0·75 cc. (= 0·75 agar growth). Care was taken to give the emulsion as far as possible on an empty stomach.

No symptoms were observed except on one occasion, when, after a dose of 0·5 cc. of a glycerine emulsion of a virulent strain, there was headache of a mild type, and some diarrhœa later in the evening. It turned out afterwards, however, that two other men living in the same hotel had also been attacked by diarrhœa that evening, and a repetition of the same dose did not cause any untoward symptoms. The result of the experiment was that there was a marked rise in the bactericidal power of the patient's serum, as is seen on the accompanying charts (XIV. to XVI.), reaching a maximum of six times normal a month after commencing treatment, and ending up at the conclusion of the experiment two and a-half times greater than normal. In this experiment, also, there

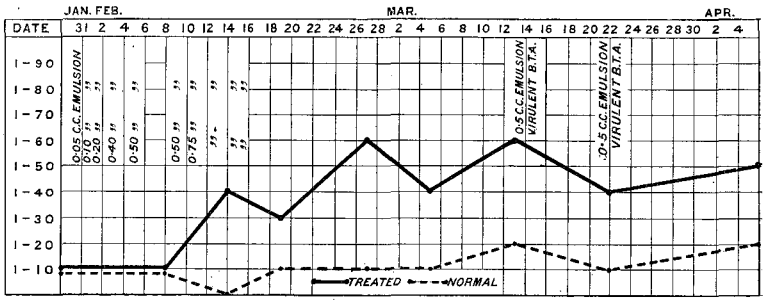

CHART XIV.—Bactericidal action of the serum of a normal man (T. H. G.) after swallowing a glycerine emulsion of *B. typhosus*, compared with the bactericidal action of the sera of two normal men.

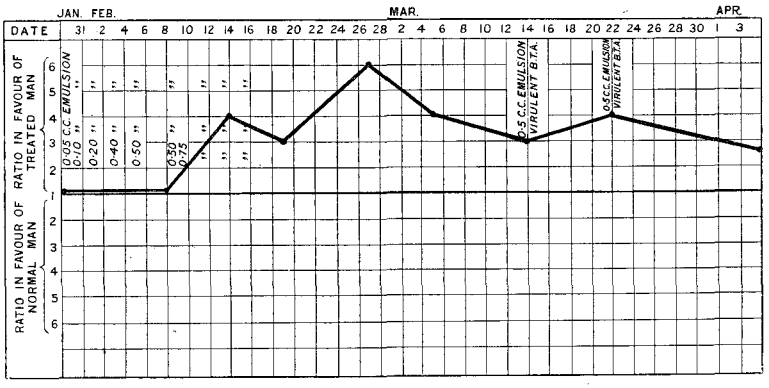

CHART XV.—Curve showing the rise and fall of the bactericidal action of the serum of a normal man (T. H. G.) after swallowing a glycerine emulsion of *B. typhosus*, the grouped sera of two normal men being taken as unity.

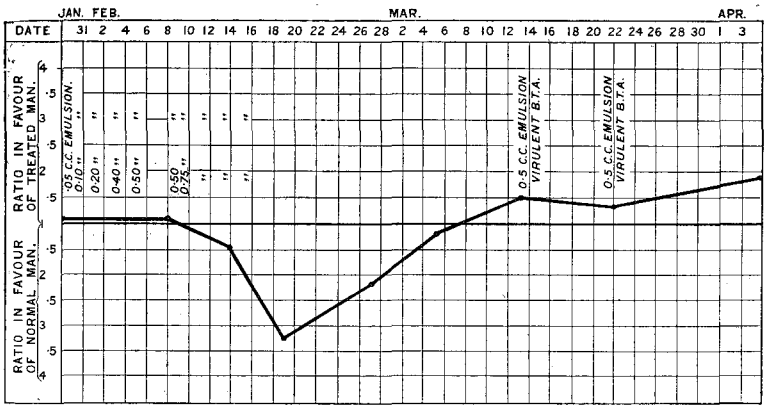

CHART XVI.—Phagocytic ratio of the serum of a normal man after swallowing a glycerine emulsion of *B. typhosus*, the grouped sera of two normal men being taken as unity.

was very little formation of agglutinins, which rose to a maximum of 1—30 on March 5th and ended up at 1—20 (incomplete reaction). The phagocytic ratio was depressed during the whole time that the emulsion was being taken and for nineteen days thereafter, it then recovered and rose above normal, where it remained until the conclusion of the experiment. There was no evidence of stimulins (except on one occasion) whilst the phagocytic ratio remained in the negative phase, and very little afterwards. In this respect the results of this experiment differ from those of the preceding one, where the evidence of the presence of stimulins in the serum was very marked. It may be that this was due to overdosing of the patient. The results of the two experiments, as well as those of a third, which was unfortunately incomplete, are promising, and further research in this direction is now in progress.

On the Duration of the Immunity resulting from the Injection of Anti-Typhoid Vaccine.

First Experiment.—The bactericidal action on *B. typhosus* of the serum of a man inoculated one year previously was compared with that of a normal man; the result showed that in the case of the vaccinated man his serum was bactericidal to typhoid bacilli in a dilution of 1—80, whilst normal serum killed in a dilution of 1—20; the serum of the vaccinated man likewise gave an agglutinin reaction in 1—200 dilution, and the phagocytic index was the same as normal.

Second Experiment.—An estimation of the anti-tropic substances contained in their blood was made on two groups of six men of the 7th Hussars, who had been inoculated four years previously, and had subsequently served in South Africa; the normal control serum was the pooled sera of six men of the same regiment who had the same service and had served under the same conditions as the inoculated men. The first group of inoculated men consisted of those men who had a severe reaction at the time of inoculation, and the second group of those who had only a mild reaction. The results are shown in the following table :—

Bactericidal Action—

Count	Serum	1—10	1—20	1—30	1—40	1—50	1—60	Control
860 millions per cc.	Normal ..	0	+(2)	+(7)	+(6)	+(11)	—	About 350 colonies
	Group 1 ..	0	0	0	0	0	+(1)	
	Group 2 ..	0	0	0	0	+(3)	0	

0 = sterile; + = growth (with number of colonies); — = not tried.

Agglutinins—
Normal = 1—10 complete
Group 1 = 1—30 not complete
Group 2 = 1—20 complete
Phagocytic Index—
Normal = 16·7
Group 1 = 16
Group 2 = 16·6

The presence of agglutinins in the normal group serum may have been due to the presence in the group of a man who had suffered from an unrecognised attack of typhoid fever. The culture with which the experiment was made was checked with other normal sera, and was not agglutinated by them in dilutions of 1 in 5 upwards.

Third Experiment.—The previous experiments were repeated, this time on the serum of an officer of the 7th Hussars who had been inoculated four years previously. The normal control serum was the pooled sera of two normal men (officers). The results were as follows:—

Bactericidal Action—

Count	Serum	1—10	1—20	1—30	1—40	1—50	1—60	Control
852 millions per cc.	Normal ..	0	+(5)	+(6)	+(2)	+(8)	+(31)	About 400 colonies
	Vaccinated	0	0	0	0	+(2)	+(6)	

0 = sterile, + = growth (with number of colonies).

Agglutinins—
Normal = *nil* in 1—5
Vaccinated = 1—30 complete
Phagocytic Index—
Normal = 10·8
Vaccinated = 9·5

Fourth Experiment.—The serum in this case was that of an officer who had been inoculated six years previously, and had never had typhoid fever. He had served in Egypt (war), Crete and the Punjaub. The control serum was taken from an officer who had never been inoculated or had typhoid fever; he had served in West Africa and South Africa (war). The results were as follows:—

Bactericidal Action—

Count	Serum	1—10	1—20	1—30	1—40	1—50	1—60	Control
920 millions per cc.	Normal ..	0	0	+(1)	+(7)	+(8)	+(17)	About 400 colonies
	Vaccinated	0	0	0	0	+(11)	0	

0 = sterile, + = growth (with number of colonies).

Agglutinins—
Normal = *nil* in 1—10
Vaccinated = 1—20 complete

It would appear, then, from the above experiments, that evidence of a bactericidal activity higher than normal and of the presence of agglutinins, can be obtained from the serum of men who have been inoculated as long as six years previously, but whether the protection that remains would still suffice to ward off an attack of typhoid fever is not yet known. The persistence of agglutinins was not expected, and is not without importance from the clinical point of view.

On the Protective Action of Different Vaccines Against Multiple Lethal Doses of a Virulent Typhoid Culture.

The virulent culture used for this experiment was one which was obtained from the Lister Institute in October, 1904. At that time it was fatal within twenty-four hours when given to guinea-pigs intraperitoneally in a dose of 0·5 cc. per 250 grammes weight. Since its receipt it had been kept in agar stab in a sealed tube. Broth cultures were made from this and tested for virulence after twenty-four hours' growth at 37° C. with the following results :—

	Weight of G.P.	Dose Intraperitoneally	Result
1	615 grammes	2·5 cc.	Died (under 20 hours).
2	465 grammes	1 cc.	Died (27 hours).
3	350 grammes	0·7 cc.	Died (under 22 hours).
4	260 grammes	0·5 cc.	Died (under 20 hours).

It appeared, then, that this culture could be relied on to kill guinea-pigs within twenty-four hours when given intraperitoneally in a dose of 0·5 cc. per 250 grammes.

Guinea-pigs were vaccinated with cultures of *B. typhosus* killed by heat at 60° C. and at 53° C., and by chloroform, the vaccine in each case being of precisely the same strength, and the dose given being 0·1 cc. per 100 grammes of guinea-pig.

First Experiment.—A group of vaccinated guinea-pigs, along with one normal one as a control, were given test doses of 1 cc. virulent culture per 250 grammes, intraperitoneally, twenty-two days after vaccination. The test culture was a particularly thin one, having been made directly from an agar stab; the result was that all the animals survived, including the control.

Second Experiment.—The above experiment was repeated, this time taking the precaution to pass the test culture once through broth so as to get a growth of average strength. The test dose of

1 cc. per 250 grammes was given twenty-seven days after vaccination. The result was that the control animal died in less than twenty-one hours, and *B. typhosus* was found in pure culture in its heart-blood. All the vaccinated guinea-pigs survived.

Third Experiment.—The test dose was given forty-three days after vaccination, the dose being 1·5 cc. per 250 grammes. The result was that all the animals survived, including the control. This was probably due to the fact that the virulent test culture had been subcultured daily for a fortnight and had thus lost a good deal of its virulence. It had not been anticipated that the virulence would disappear so rapidly during a series of sub-cultures.

Fourth Experiment.—The test dose was given eleven days after vaccination, the dose being 1·5 cc. per 250 grammes. The culture used was one made after one passage through broth from an agar stab (in which the bacterium seems to retain its virulence practically unaltered for some months). The result was that all the animals died within twenty hours, except the one which had been vaccinated with a culture killed by chloroform; this one survived. Typhoid bacteria were recovered in pure culture from the heart-blood of the animals which died.

Fifth Experiment.—In this the test dose was reduced to 1 cc. per 250 grammes, in the hope that one might find out some further differences in the protective properties of the three different vaccines. The dose was given twelve days after vaccination, and all the vaccinated animals survived; the control died within twenty hours, and *B. typhosus* was recovered in pure culture from its heart-blood.

Sixth Experiment.—In this the test animals were given two doses of $\frac{2}{10}$ cc. vaccine per 100 grammes at an interval of eleven days, this followed by a dose of four times that quantity, the idea being to see if one could establish any differences in the protective powers of the different vaccines by raising the immunity to a fairly high level. The test dose of 2 minimum lethal doses was given thirteen days after the last dose of vaccine; the result was that the control died in under twenty hours and the vaccinated animals all survived. *B. typhosus* was recovered in pure culture from the heart-blood of the control.

Seventh Experiment.—The animals were vaccinated in the same way as in the last experiment, and a test dose of 10 minimum lethal doses was given fourteen days after the last dose of vaccine, the control getting a single lethal dose. The result was that all the vaccinated animals survived, whereas the control died within twenty-four hours and typhoid bacilli were recovered in pure culture from its heart-blood.

So far as the attempt to discover differences in the protective value of the three different vaccines was concerned the results of these experiments are inconclusive, but they show that a dose of $\frac{1}{10}$ cc. per 100 grammes of a culture killed by any of the three methods which were tried, will protect a guinea-pig against twice a lethal dose of *B. typhosus*, and that, if one repeats the doses of vaccine, one can protect a guinea-pig against at least ten times a lethal dose.

Addition of Antiseptics to Vaccine.—The following experiments were made to determine the smallest quantity of lysol which could be relied on to prevent the growth of accidental contaminations, should any occur in the vaccine.

Broth containing various quantities of lysol was inoculated with (1) *Staphylococcus aureus*, (2) *Bacillus typhosus*, (3) *B. mesentericus*, (4) *B. tetani* (in this last case glucose broth was used and the cultures made anaerobic). Measured volumes of the infected lysol broth were taken at intervals and mixed with melted agar (at 43° C.), and note was taken of the number of colonies which grew as compared with control specimens taken immediately after infecting the lysol broth. The results were as follows:—

(1) *S. aureus* increased in $\frac{1}{20}$ per cent. to $\frac{1}{10}$ per cent. lysol-broth and failed to grow in $\frac{1}{5}$ per cent. lysol-broth; at the end of three days this last was sterile.

(2) *B. typhosus* behaved in the same way as *S. aureus*.

(3) *B. mesentericus* and *B. tetani* had failed to grow in $\frac{1}{20}$ per cent. lysol-broth after three days' incubation.

It appeared, then, that $\frac{1}{5}$ per cent. lysol is quite sufficient to prevent growth of any of the organisms tried, and in the vaccine which is now issued $\frac{1}{4}$ per cent. lysol is added, leaving a margin for safety.

Observations were made on the fate of any organisms accidentally added to a bottle of vaccine containing $\frac{1}{4}$ per cent. lysol, capped in the usual way and kept at 37° C. The organisms used were *Staphylococcus*, *Streptococcus*, *Torula* and *B. subtilis;* the result was that not only did none of the organisms grow, but at the end of five days the vaccine had purified itself completely. Another experiment of the same kind where, among other organisms, *B. tetani* was used, gave the same result. In a third experiment the vaccine was contaminated with saliva; it was found sterile at the end of seven days. It appears, then, that with reasonable care in handling, a vaccine containing $\frac{1}{4}$ per cent. lysol can be trusted to be free from injurious contaminations.