THESIS

ON THE

SERUM DIAGNOSIS OF TYPHOID FEVER.

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INTRODUCTION.

During the last two years, no subject has occupied the attention of the medical profession, more than the sero diagnosis of typhoid fever.

To Dr Widal belongs the honour of having made this discovery. On 26th June 1896 he announced that he was able, by an easy method, to diagnose enteric fever, by simply watching the action that blood sera (taken from patients suffering from typhoid) had upon a culture of Eberth's bacillus in bouillon.

Although Dr Widal holds the honour of having made this discovery of rapidly diagnosing typhoid fever, it must still be kept in mind that many observers for some years previously had been studying the question of immunity in animals; and their work undoubtedly led Widal to extend his observations to the human subject. Those observers had been studying the action of sera taken from immunised animals on cultures of the organism in bouillon with which these animals had been immunised.

It will be interesting, as well as instructive, if one gives here a fairly accurate account
of the work done by these gentlemen prior to the
discovery of M. Widal.

In 1889, MM. Charrin and Roger, experiment-
ing with the bacillus pyocyaneus, made the following
discovery:-

If serum be taken from an animal immunised
against the bacillus pyocyaneus, it is found to have
a specific action against this particular organism.

Their experiments were performed on fourteen
animals (7 immunised and 7 non-immunised). Immunity
was brought about in seven of the animals by repeat-
ed inoculations, under the skin, of small doses of
the bacillus pyocyaneus. It was of no consequence
whether the inoculations were performed with dead
or living cultures (a), the result of immunity being
the same in both cases.

(a) If one wishes to use dead bacilli the best
method of destroying their vitality is to
place the culture in a vessel containing
cotton wool saturated with chloroform,
and to leave the culture thus exposed to
the vapour of chloroform for two or three
days. This kills the organisms, but at
the same time does not interfere with
their toxic action. Pfeiffer uses this
method in all his experiments which will
be referred to later.
In the case of the immunised animals, from two to twelve days after the last inoculation, blood was drawn from the carotid artery, which was received into sterilized glass vessels, these were placed aside for 48 hours in an ice chamber to allow the serum to separate. The serum was then decanted into sterilized test tubes.

Serum was obtained from the non-immunized animals in the same way.

We have now before us two sets of test tubes, one containing serum taken from the immunised animals; the other, serum from the non-immunised animals. Each set was then inoculated with a small quantity of a culture of bacillus pyocyaneus in bouillon and placed in an incubator at 38°C.

On examining these sera after 24 hours the following changes were to be observed:

In the case of the sera taken from the non-immunised animals extreme turbidity of the fluid was noticed which increased during the next few days, and on examination under the microscope, the bacilli were actively motile. On the other hand, the fluid in the test tubes, containing the sera of the immunised animals, was clear and transparent and a dis-
Distinct sediment was seen at the bottom of each tube. This sediment consisted of clumps or masses of bacilli, aggregated together, which were perfectly immobile, as revealed by the microscope. When these tubes were shaken, uniform turbidity resulted but on allowing them to stand for a short while the precipitate again began to sink to the bottom of the tube.

Thus a distinct difference was noticed in the behaviour of immunised and non immunised serum on the bacillus pyocyaneus.

On making a culture of the bacilli from the immunised serum on agar agar, the culture was far less abundant than that grown from the non immunised serum.

MM. Charrin and Roger refer to other changes which were noted between the bacilli but these need not be alluded to here.

In 1890 Behring and Nissen in an article of theirs termed the "Bactericidal properties of various kinds of blood sera," describe the action that sera of highly immunised guinea pigs have on the vibrio Metchnikovii.

They say that although the vibrio Metchni-
kovii easily kills guinea pigs and pigeons, at the same time a high degree of immunity can be obtained in these animals with the above vibrio. On taking 1 c.c. of blood from a pigeon, which had succumbed to the Vibrio Metchnikovii, and injecting it into two guinea pigs which had not been immunised, it was found that they soon died; on the other hand if the same quantity of pigeon's blood be injected into immunised guinea pigs death does not follow, and the sera of these animals show a strong bactericidal action against the vibrio.

They have shown that the sera of animals which are easily immunised with the comma bacillus of cholera, Fraenkel's pneumococcus, anthrax, and the Vibrio Metchnikovii all show a very marked and active bactericidal action against their respective organisms; also that animals that have a weak power of immunising themselves against these bacteria show hardly any bactericidal action in their sera.

They conclude by saying that the bactericidal substances in the sera of the above variously immunised animals must differ in each case as they only have an action on the respective bacteria with which immunisation has been produced.
In 1891 Metchnikoff in one of his articles in the "Annales de l'Institut Pasteur" disputes somewhat the observations of Behring and Nissen. He says that the action they observe in the case of immunised animals is not due to bactericidal substances in the blood but is due to phagocytosis.

His first experiments were performed with the Vibrio Metchnikovii; and the inoculations were made in the anterior chamber of the eyes of immunised and non-immunised guinea-pigs.

Soon after inoculation ophthalmia resulted in both cases, a good deal of oedema was present and the cornea became dim. A drop of exudation was now withdrawn from the anterior chamber in each set of animals and examined under the microscope. That taken from non-vaccinated guinea-pigs revealed the presence of large numbers of the vibrios but hardly any leucocytes were to be seen; whilst in the vaccinated guinea-pigs vibrios were present but leucocytes were also to be found in abundance, and they also contained many of the organisms in their interior. Cultures could be obtained from the exudation withdrawn from the eyes of the vaccinated guinea-pigs as late as 8 days after the inoculation.
but after this date it was generally found that all the vibrios had been killed and Metchnikoff says that this death is due to the leucocytes.

Those guinea-pigs which were not vaccinated succumbed soon after the inoculation was made.

Metchnikoff further states that after the subcutaneous inoculation of these vibrios, in the case of vaccinated guinea-pigs, abundant cultures can be obtained, and that they have only to get accustomed to live in the organism of an immunised animal. He therefore thinks that Behring and Nis-sen are wrong, because if the action they describe was due to bactericidal substances in the blood all the vibrios ought to be killed.

Having dwelt at some length on the question of immunity, Metchnikoff speaks of further observations he has made while experimenting with the above vibrios, which are as follows:–

If serum taken from one of the immunised guinea-pigs was inoculated with a culture of the Vibrio Metchnikovii, certain changes were to be noted. The vibrios become immobile and form into "clumps" or "packets," which sink to the bottom of the test tube, leaving the fluid above clear and transparent. On the other hand, if serum taken from a non-immunis-
ed guinea-pig was inoculated; the vibrios do not form into clumps, but remain free and mobile and develop as in ordinary media.

He adds towards the end of his paper:-
"I have also found that the pneumo-coccus forms in the serum of vaccinated rabbits, bundles of cocci, which is not the case if the rabbit is not vaccinated."

The next step was made by Pfeiffer in 1894, who states that cholera immunised animals only have an action against the cholera vibrio and that the serum of these animals has no more action against other species of organism than the serum taken from non-immunised animals.

His experiments differ somewhat from those of the former observers, in that he always made his injections of cholera vibrios into the peritoneal cavity of guinea-pigs, which had been previously immunised; or else into non-immunised guinea-pigs, but in this case the injection was first mixed with some serum taken from an immunised guinea pig.

After a short period the exudation was then drawn off by means of fine glass pipettes.

Pfeiffer in his article proceeds by stating
that "The serum of a normal guinea-pig has almost no bactericidal influence against virulent cholera cultures, in fact increase of the number of organisms is to be noted; on the other hand, the effect of the serum of an immunised guinea-pig on a cholera culture is marked, causing death of the vibrios, with destruction of them, if the injection be not too great."

Lazurus Wasserman and Pfeiffer found that a small quantity of serum taken from a cholera conval-escent, and injected along with an emulsion of cholera vibrios, into the peritoneal cavity of a non-immunised guinea-pig, caused the death of the vibrios in a short space of time.

Pfeiffer further states that no bactericidal substances exist in a normal guinea-pig, but that they are produced after injection has been performed, and that passive immunity does not depend on the transference of bactericidal substances in the serum but may be due to phagocytosis, as suggested by Metchnikoff. As we have already seen, Metchnikoff holds that the serum of an immunised guinea-pig acts as an irritant to the leucocytes, causing them to swallow up and destroy the organisms within their interior.

Pfeiffer doubts this, and believes that
phagocytosis is only an accompaniment to the destruction of the vibrios.

In detail he performed his experiments as follows:

1/3 of a culture of dead cholera bacilli (a) is injected into a guinea-pig weighing about 600 grm.

Six days afterwards, half an ordinary platinum loopful(b) of an active virulent cholera culture is injected. At various intervals serum is drawn off from the peritoneal cavity by means of fine glass pipettes and examined in hanging drop preparations.

Changes to be noticed:

After 1 hour. There are a great number of moving vibrios but already many have become immotile. Spherules, coccus-like bodies, leucocytes, and degenerated forms of vibrio may be seen. The leucocytes contain in their interior many of the coccus-like bodies and round about them vibrios are scattered.

In about 2 hours the motility of the vibrios ceases,

(a) The culture is killed as noted in footnote to page 2.

(b) For accuracy Pfeiffer uses a standard size of platinum loop, which, when containing a portion of the culture, should weigh about 2 m.grms.
and the coccus-like bodies increase in number. After 4 or 5 hours the process ceases.

If the immunisation of the guinea pigs is carried out to a higher degree by further injections of the live cholera vibrios, the action above described takes place far more rapidly and the degeneration of the vibrios is more complete; also very few, if any, leucocytes are to be seen: Pfeiffer has thus watched this reaction taking place without the aid of leucocytes, and on this account holds that Metchnikoff is wrong; that degeneration is not due to phagocytosis, but to some other power, such as a bactericidal substance in the serum.

He says the action may be accounted for in the following ways:—

1. The presence of an electrical stream.
2. Bactericidal substances produced on injection which were not present before.

Kossel's theory he also disputes, as he injected a highly immunised cholera guinea-pig with cholera and Nordhafen Vibrios. If Kossel's theory were correct, then both forms of organism ought to be de-
stroyed. On the contrary the Nordhafen Vibrios escaped destruction while the cholera vibrios immediately underwent the above changes. Pfeiffer then thought that the action might be due to the cholera vibrios possessing a weaker power of resistance than the Nordhafen Vibrios, so he repeated the experiment in the reverse order (that is to say he used a Nordhafen immunised guinea-pig) with the result that this time the Nordhafen Vibrios were destroyed, while the cholera vibrios escaped.

From these experiments Pfeiffer hoped he would be able to establish an easy method of diagnosing between the various allied species of cholera vibrio.

Metchnikoff says that the above described coccus-like bodies give an abundant culture on agar. He finds in his experiments that in hanging drop cultures, polynucleated, mononucleated, and eosinophilous cells are all surrounded by the vibrios while the red cells are quite free. He attributes this action to a power the leucocytes have of attracting organisms. Before the organisms can reach the leucocytes, they have to swim in the serum, and it can be noted that they only lose their motility in the close neighbourhood of them.
Metchnikoff explains "Pfeiffer's phenomena" as follows:

"The phagocytes of vaccinated guinea-pigs elaborate in their interior substances capable of killing the vibrios. In the state of hyper vaccination these substances become so abundant that they can easily escape from the cells, and theoretically speaking one ought to be able to bring all phagocytes, and other cells to secrete their bactericidal substances; and so transform the intracellular destruction of microbes into a extracellular destruction."

Further experiments of Pfeiffer show that immunised serum if kept in the dark and in an ice chamber, retains its bactericidal influence for months. On the other hand, if the serum is raised to a temperature of 70°C. the bactericidal influence diminishes, and is lost if raised to a temperature of 100°C.

The live vibrios which Metchnikoff says he has found in immunised guinea pigs some days after inoculation, Pfeiffer also describes, and he explains this by saying that these vibrios may have been protected by the protoplasm of the cellular elements of the leucocytes, thus escaping the influence of
the bactericidal substances in the serum.

From the preceding résumé of the work done, it will be noticed that two opinions exist regarding Pfeiffer's phenomena.

(1) Those who hold with Metchnikoff, the cellular theory of phagocytosis, and

(2) Those who hold with Pfeiffer, the humoral theory – that the reaction is due to some bactericidal substances produced in the blood serum.

Metchnikoff and Bordet produced in vitro Pfeiffer's phenomenon by mixing cholera vibrios with some immunised serum and adding to it leucocytes taken from the peritoneal cavity.

Bordet, in his paper, continues by saying:—

"We have shown that the cholera vibrio introduced into a mixture of preventive serum and defibrinated blood, taken from a normal guinea-pig, rapidly transforms the leucocytes into rounded
grains or coccus-like bodies. This change is produced in vitro as completely as in the organism itself.

The following are the deductions which M. Bordet arrives at:-

That the serum of highly immunised animals does not possess any bactericidal substance peculiar to it.

That these substances exist in normal serum, but cannot come into activity until mixed with the preventive substances which only exist in vaccinated animals, and therefore does not show any reaction except on very attenuated organisms.

There is no need for me here to discuss the value placed upon either theory, as I hope to allude to it again when mentioning more fully the action of typhoid serum on Eberth's bacillus.
In the same year as Pfeiffer - Issaeff and Ivanoff, working with a vibrio, which the latter had discovered in the stools of a typhoid patient, describe the same reaction that Charrin and Roger mention in their paper with regard to the bacillus pyocyaneus. Pfeiffer regards this organism as a true cholera vibrio as it gives his reaction with cholera immunised serum.

After Pfeiffer had been working with the cholera vibrio and its reaction to cholera immunised serum with a view to establishing a method by which it would be easy to make a differential diagnosis between allied species of this organism, he next turned his attention to the typhoid bacillus for the same purpose. He and Kolle together, lay stress on the importance of having a proper nutrient medium for the growth of Eberth's bacillus. If the medium is not suitable, then on examining some of the culture under the microscope, many of the bacilli are found to be motionless, showing that they are already dead.

On injection of a virulent culture into the abdominal cavity of an immunised guinea-pig, changes are seen which are similar to those produced by cholera immunised animals on the cholera vibrio. On
withdrawing some exudation the bacilli become thinner, and the rods break up into fragments, and they become motionless, granulation also takes place but is not so rapid as in the case of cholera. They find that serum taken from typhoid patients has no effect on other organisms, such as the coli communis.

They conclude from their experiments that antitoxic substances are present in the serum of typhoid convalescents, and typhoid immunised guinea pigs, which have a bactericidal effect on the Eberth bacillus only, and not on its allied species. That the bactericidal substances (antikörper) remain inactive in the serum of normal guinea-pigs and only become active at the time of infection.

Pfeiffer and Kolle and other observers quite overlooked the phenomenon of "clumping" which occurred with Eberth's bacillus when treated with typhoid serum. Bordet was the first to point out this reaction but he does not attribute any significance to this phenomena.

Durham in his paper on the "Action of the serum of immunised animals" repeats to a large extent the work done by Pfeiffer on cholera immunity. He however points out a means of estimating the degree
of reaction of a cholera serum with the aid of litmus solutions, and thus the reducing power of the organisms can be noted.

The solution he has found most useful is as follows:–

- 2% Sugar of Milk.
- 1% Peptone.
- 0.5% Sodium Chloride
- 4% Saturated neutral Litmus Solution.

If one is testing the power of reaction of the cholera vibrio, on various cholera sera, it is often hard to note slight differences of turbidity, but, with this colour test, minutest changes can be noted.

Durham says he has been unable to produce clump formation of organisms except with the sera of immunised animals.

He further shows by his numerous experiments, that "different kinds of cholera vibrios all give a positive reaction with their own serum," and also that the more virulent forms give a reaction with the less virulent, but that the less virulent have no effect, or very little, on the more virulent.
Thus the Cholera "Ehm" serum reacts with all the forms of cholera vibrio used, but the Cholera "Indien" serum, which is a less virulent form than the "Ehm" serum, only shows a very slight action on the "Ehm" vibrio.

These observations, as will be seen, do not completely correspond with those of Pfeiffer. Having worked with the cholera vibrio and its allied species, he next turns his attention to Eberth's bacillus, and the bacillus coli communis; and states that they both react with their respective sera, but not on each other.

From his experiments it will be noted that the sera of allied species react with those allied species, but that organisms widely separated in their morphology do not interact in this manner.

Gruber and Durham were the first to give the name of "agglutinins" (Verkleber agglutinine) to those specific substances in the blood sera of immunised animals which cause the bacteria to run together and form clumps.

Gruber and Durham, working conjointly with the cholera and typhoid sera, in detail, perform their experiments as follows:-

Given a culture to be tested, they take
1 loopful (2-4 m.grm.) and mix in $\frac{1}{3}$ c.c. of bouillon; this solution is added to 10 m.grm. of highly immunised serum in $\frac{1}{3}$ c.c. of bouillon and is then watched macroscopically and microscopically to see if perfect agglutination occurs.

From the foregoing experiments performed by several observers, it will be seen that the sera of immunised animals possesses certain definite qualities when mixed with the specific organism with which they had been immunised.

It was this fact which led M. Widal to experiment with sera taken from patients suffering from typhoid fever, to see whether they exhibited any of the above definite qualities on the Eberth bacillus.

He was amply repaid for his observations, as he found that these sera had a very marked effect on the Eberth bacillus when brought in contact with it; exhibiting the same qualities as those described by Pfeiffer, Metchnikoff, Bordet, Durham, etc., while experimenting with sera from immunised animals.

He thus showed that this reaction might be looked for during the period of infection, and not only when a state of immunity had been reached.

We can now turn our attention to the study of the various methods of performing this test.
I. M. Widal's original methods as announced by him on the 26th June 1896 at a Meeting of the "Société Médicale des Hôpitaux.

1. Having rendered thoroughly aseptic a portion of skin on the front of the fore arm of a patient suffering from enteric, he punctures a vein and draws off a small quantity of blood (about 3c.c.). The serum is allowed to separate, and then is decanted into a sterile test tube. To this is added from 10-15 parts of neutral bouillon solution; the mixture is then inoculated with Eberth's bacillus, and placed in an Incubator, at 37°C. for 24 hours. After this time, on examining the tube, the fluid is quite clear, or slightly dim, with a floccular deposit at the bottom. If the tube is allowed to stand for 2 or 3 days, the reaction becomes more marked. On examining the precipitate under the microscope, it is found to consist of masses of agglutinated bacilli, which are quite immotile.

In performing this test it is better always to have a control tube, so that more accurate
results can be obtained. This is done by simply inoculating a tube containing bouillon, and serum, from a patient who has never had enteric fever.

In this case, on examining the tube after it has stood for 24 hours, the fluid is found to be turbid in its entirety, no precipitate having taken place, and the supernatant liquid does not become clear, as when serum is used which has been taken from a typhoid subject. On examination of a drop, under the microscope, it is found to contain active and freely motile bacilli, and no masses, or "clumps" can be seen.

2. Instead of inoculating a tube containing bouillon and serum taken from a typhoid patient, as described above, Widal also used to make his Eberth culture in neutral bouillon, and allow this to incubate for 24 hours, and at the end of that period, to add the serum to it taken from his typhoid patient, in the proportion of 1 to 10, 15 or 20, thus obtaining the same result.

3. A third and quicker method was as follows:— Wash the pulp of a finger, or lobe of an ear and render the surface thoroughly dry and aseptic. Prick
the surface with a needle or some sharp instrument; obtain a few drops of blood and allow it to flow into a small tube, something after the fashion of a thimble; allow the blood clot to separate from the serum; then take one drop of serum and mix with 10 or 15 drops of a culture of Eberth in bouillon, and examine this fluid in a cell, under the microscope.

If the serum be that taken from a typhoid patient, the motility of the bacilli ceases and they run together into masses, forming small islands. Isolated motile bacilli may be noticed swimming about between the clumps, but, if the process is allowed to proceed for a longer period, these also become immotile and adherent to some of the clumps.

Widal found that the above reaction of the Eberth bacilli to typhoid serum, took place much more quickly in the case of typhoid immunised animals, than in patients suffering from this disease, and he says that more serum is required to bring about the action. The greater the immunity of the animal, the more active is its serum.

He tested the blood of healthy people, and of those suffering from various diseases, and men-
tions that all have given a negative result, that is to say the bacilli remain isolated and motile. He also tried the action of typhoid serum on coli bacilli with the same result.

M. Achard, reporting on cases that came into hospital under his charge, says that he has found the test infallible and that it was of great service in helping to diagnose those cases where the symptoms were obscure, or where patients were suffering from very mild attacks of enteric fever.

M. Widal lays great stress on the importance of examining the Eberth culture with which one is working before performing the test, as in many cultures, where the bouillon has not been prepared properly, many died, and therefore immotile bacilli may be found and spurious clumps noticed. If such a culture were used it can be easily seen that a positive result might be noted where no typhoid fever existed.

On experimenting with various fluids, Widal found that the serum taken from vesicles, urine, tears, saliva, and milk of patients suffering from enteric fever, in every case gives the reaction but that it takes a much longer period than when the serum of the blood is employed.
In the case where the milk was examined, the patient was suckling her child, but the reaction could not be obtained in its blood.

Experimenting with dried serum to see how long it retained its agglutinative properties, or if it had any at all, he found that typhoid patients invariably gave the test, while in those suffering from other diseases no reaction could be obtained. Heating the serum destroyed its agglutinating power.

On testing for the date of the appearance of the reaction, he found it present as early as the 5th, 6th and 8th days of the disease, and that the test became more marked as the malady progressed.

He next turned his attention to the agglutinative property of typhoid serum, to see if he was able to obtain any one constituent element which gave the reaction. If the serum globulin, from a typhoid serum, be separated by precipitation with a saturated solution of sulphate of magnesium; one finds no reaction takes place with the Eberth bacillus, but that if the globulin, which has been separated, be brought in contact with a solution of typhoid bacilli in bouillon, the reaction at once occurs. Fibrinogen also shews the agglutina-
tive power, but plasma which has been deprived of fibrinogen and serum globulin gives a negative test.

In the case of milk taken from a typhoid subject, the casein and lact-globulin give the reaction, while the lact-albumin fails.

Lastly Widal wished to find out how long after the disease the reaction could be obtained. In one case he found the blood give the reaction as late as 3 years and in another as late as 7 years.

Achard tested the blood of a foetus, obtained from a case of abortion, where the mother was suffering from typhoid fever, and states that he was unable to obtain any reaction. Recently a similar case was admitted into the R. S. M. Hospital and the reaction of the foetus' blood was most marked. Abundant cultures of the Eberth bacillus were also obtained from the spleen and other organs.

Batty Shaw, in the Lancet of August 28th 1897, mentions a case where a child five weeks after delivery, showed in its blood the Widal reaction. The mother had contracted typhoid during gestation.
II. Wyatt Johnston's
Dry blood Method.

Although Widal had found that dried serum and blood of enteric patients exhibited the agglutinative properties of fluid serum, he did not make practical use of this method. Wyatt Johnston was the first to put on record a series of cases tested by this method, which may be described as follows:

Prick the finger or lobe of the ear as described before, and allow a drop of blood to fall on a piece of clean non-absorbent paper. When this has dried, it can be examined at leisure in the laboratory, by simply moistening the blood with a drop or two of bouillon. Remove some of this to a cell slide and add a small quantity of a culture of Eberth's bacillus in broth, then examine under the microscope with an ordinary high power lens.

The superiority this method has over that described by M. Widal, is that any practitioner who has not the necessary appliances to hand, is able to transmit by post a sample of blood to a central research laboratory. This method does not require immediate examination, and aseptic precautions are not
necessary as when fluid serum is employed.

Wyatt Johnston claims that this method is as accurate in every detail as the others described by Widal.

Cholera blood treated in the same way gave the reaction with the Cholera vibrio.

Out of 143 cases of suspected typhoid he obtained a positive result in 123.

Decisive positive results on first examination 118
Do. Do. second Do. 5
Do. negative results 20

(These cases were afterwards proved to be some disease other than typhoid.)

The conclusions he arrives at from using dry serum are as follows:

I. A positive result can often be obtained as early as the 2nd day of the disease.

II. A complete reaction is rarely delayed after the fifth day.

III. Dry typhoid serum kept for 60 days still gives the reaction, and he believes that for this reason the dry method might be applied to medico-legal cases.

IV. Cholera dry serum gives a similar reaction.
III. Sheridan Delépine's Method.

I must here take the opportunity of thanking Dr Delépine for his great kindness in having sent me reprints of all his papers. No one could fail to obtain a thorough knowledge of the serum diagnosis and its technique, after reading his paper in the Medical Chronicle. I may also mention that the method I have adopted, of arranging the statistics of cases at the end of this thesis, has been partly obtained from that given by him in the Lancet of December 12th, 1896.

He performs the test as follows:–

Obtain a drop of blood from the finger (taking all necessary antiseptic precautions), and draw it up into a special pipette, designed by himself, by applying suction after breaking off its point.

The pipette before being used is kept sterile by having the one end hermetically sealed and the other plugged by cotton wool, the whole being raised to a suitable temperature in order to destroy all organisms.

Having sucked up the drop of blood into the instrument, the point is again sealed, and the constricted portion near the mouthpiece is drawn out in a bunsen flame and also sealed. The tube con-
1. Modified Pasteur's pipette. A & B points which are sealed in a flame after the capsule C has been filled with blood.

2. Sealed Blood Capsule showing clot and serum separated.
taining the blood is laid aside and the serum is allowed to separate from the clot.

When one wishes to examine the reaction of the serum; the point of the pipette through which the blood was drawn is broken off and a drop is blown out, on to a cover glass, by heating the opposite end.

To this drop of serum is added a quantity of Eberth's bacillus in bouillon, in the required proportions, and the whole is examined in a cell under the microscope.

If it is desired to preserve the remainder of the serum in the pipette, all that is required is that the point be again hermetically sealed in a flame.

Dr Delépine lays great stress on one point, namely in sealing the point of the pipette care must be taken not to heat the blood as this destroys the agglutinative action of the serum.

Photographs of the pipette employed, with a description may be seen on the accompanying page.
IV. Grünbaum's Method.

Dr Grünbaum, having obtained his blood from the patient, draws it up into a U-shaped capillary tube. This is then centrifugalised and broken off at the junction of the serum with the red corpuscles.

The serum is blown out on to a glass slide. A graduated capillary tube of equal calibre is now taken, and the serum is drawn up to the 1st figure, then bouillon is added till the whole reaches figure 17. This is blown out on to another glass slide and thoroughly mixed. A drop of this last is mixed on a cover glass, with another drop of typhoid emulsion, as nearly as possible of the same size, and the whole examined in a cell slide under the microscope.
V. A. E. Wright's Sedimentation and dead bacillary Methods.

A. Sedimentation Method.

Blood having been drawn up into a capsule, (something after the shape of that seen in Fig.1), the two ends are sealed in a flame.

The serum and clot having separated, both ends are again broken open, and the serum drawn off by means of a capillary tube, up to the mark c. Fig. 3. The serum is now blown out into a watch glass and the necessary dilutions are made, by drawing up normal salt solution with the capillary tube (Fig.3).

If, for instance, a 10-fold solution is required - normal salt solution is drawn up to mark c. nine consecutive times; each time blowing it out into the watch glass containing the serum. Thus it will be seen that one is able to make a 10-- 15-- or 100-fold dilution.

Having diluted the serum, and mixed it well with the salt solution, draw up a small quantity into the sedimentation tube - say up to the mark d. fig. 4. Tilt the point of the tube upwards and allow a bubble of air to
enter, the diluted serum will now occupy the space between the letters E. F. The serum can be prevented from running out of the instrument by compression of the indiarubber tube on the other end, through which suction is made.

With the diluted serum occupying the space E. F. and the bubble of air the space F. M., the point of the sedimentation tube is now immersed in an emulsion of Eberth's bacillus, in bouillon, and the fluid drawn up to the mark D. The whole is then allowed to run into the chamber H. and is there thoroughly mixed. The mixed fluid is now blown back into the stem, and the end marked M. sealed in a flame.

This instrument is placed in an incubator and sedimentation is looked for at the end of 24 hours.

If our first dilution was 20, our second will naturally be 40, when we have added the emulsion of typhoid bacilli, or again if our first was 50, our second will be 100.

On removing the tube from the incubator, if sedimentation has taken place, a fine pre-
cipitate will be noted at the lower end of the stem, next the sealed end, while the supernatant fluid becomes quite clear. On the contrary, if sedimentation is not going to take place the whole fluid remains turbid and no precipitate occurs.

Professor Wright has used this method for diagnosing both Typhoid fever and Malta fever. In his experiments he always uses a control tube, containing normal serum, mixed with bacterial emulsion, so that slight degrees of difference can be more easily noted.

B. Dead Bacillary Method.

Widal was the first to point out that the phenomenon of agglomeration, which can be demonstrated by the action of typhoid serum on the bacillus typhosus, is equally well obtained by the use of a culture of bacteria which has been exposed to a temperature sufficient to kill the bacilli.

Professor Wright and Surgeon-Major Semple were the first in this country to put on record statistics showing the accuracy of this observation. They have also proved it
to be as accurate in the case of the micrococcus melitensis of Bruce, when it is brought into contact with the serum taken from a patient suffering from Malta fever.

Professor Wright prepares his emulsions of dead bacteria as follows:

Emulsions in bouillon, of fresh agar cultures of the respective bacteria (bacillus typhosus and micrococcus melitensis of Bruce) are prepared and drawn up into small glass capsules similar to those used for bacterial vaccines.

These capsules are exposed in a hot water bath, to a temperature of 60°C. for 10 minutes, and then laid aside for from three to six weeks. After this period they are thoroughly shaken up and can be employed as if he was using a live emulsion.

If dead bacilli are used in the sero-sedimentation tubes, care must be taken not to mistake the fine precipitate which occurs with these organisms, with the true floccular precipitate that occurs when agglomeration
   A & B - points at which it is sealed when filled with blood.
2. Sealed Blood Capsule, showing blood clot and separated serum.
3. Capillary Pipette, for draining off serum from capsule. C. - graduation mark.
4. Sedimentation Tube.
   G to E - Diluted serum.
   D to F - Emulsion of bacilli & bouillon.
5. Sedimentation Tube, showing position of the mixed serum & emulsion before sealing the end.
6. Reaction, as seen after incubation. White floccular precipitate, supernatant fluid clear.
7. Control tube, showing no reaction. Note the even turbidity of the fluid.
takes place.

Out of 21 experiments performed, the results obtained with the dead bacilli correspond accurately with those obtained with the living bacilli.
The method I have employed throughout all the observations made by myself, is a modification of Grünbaum's which I first learnt from Dr. Muir.

Having pricked the finger, or lobe of the ear, a drop of blood is drawn up with an ordinary Zeiss' leucocytometer, as high as the figure 1. marked on the stem just below the bulb. This is diluted by drawing sterilised bouillon up to mark 11. The mixture is well shaken together to prevent coagulation of the blood, and the whole is then blown out into a U-shaped capillary tube. The dilution of the serum is now 1 in 10. Having filled the U-shaped tube it may be centrifugalised so as to separate the red cells which collect at the bend.

To make the emulsion of bacilli, I take an ordinary watch glass, and place in it about 1½ c.c. of bouillon, with it I mix thoroughly a platinum loopful of an active Eberth culture, not more than twenty hours old.

Great care should be taken on making this emulsion, because if any masses of bacilli are left they might be transferred later to the cell slide and be mistaken for clumps formed by the action of the serum, thus giving a wrong result.

The emulsion having been prepared, a quantity is drawn up with a fine glass pipette and two drops
Photograph of Instruments used in Dr Muir's modification of Grunbaum's method.

1. Needle for pricking finger.
2. Zeiss' Leucocytometer.
   Blood drawn up to fig. I, Bouillon added to fig. II.
3. Watch Glass, containing emulsion of bouillon and typhoid bacilli.
4. U-shaped capillary tube, 1/16 inch in diameter.
5. U-shaped tube, showing serum centrifugalised with red corpuscles collected at the bend.
   Dilution 1 in 10.
6 & 7. Capillary Pipettes for drawing up serum and emulsion.
are allowed to fall on the centre of the cell slide. To this is added one drop of the diluted serum, which may be drawn up out of the U-shaped tube by means of another fine glass pipette. The whole is now mixed together with a platinum needle, a coverglass applied, and the preparation examined under the microscope. The proportion of the serum to bouillon is 1-30, and this is the dilution that I have used almost entirely in the series of cases quoted at the end of the paper.

Another, rather rough method of performing the test, is as follows:-

Having pricked the finger of a patient allow a small quantity of blood to flow into a small glass cylinder. Put this aside, and let the serum separate from the blood clot. When this has occurred take a platinum loopful of serum and place it on a clean coverglass. With this mix ten platinum loopfuls of an active Eberth culture in bouillon (the same platinum loop being used in each case). Mix thoroughly, invert the coverglass on a hollow slide, and examine under the microscope as a hanging drop preparation. If one only has at hand a slanting agar agar culture of Eberth's bacillus; generally at the bottom may be noticed a small quantity of bouillon which has exuded from the medium, and has washed
down with it a number of the bacilli. Ten platinum loopfuls of this may be employed instead.

Of course, as has already been mentioned, this is a very rough and ready method; but it is of service especially in those cases which give a negative result, because if no result is to be obtained by this means, where one is employing a serum diluted 1 in 10, it is quite certain no result will be noticed with the more accurate method just described, where a serum, diluted 1 in 30, is employed.

It can also be used as a preliminary test before proceeding with the more accurate methods.

In using Dr. Muir's modification of Grünbaum's method, one always has left over a quantity of diluted serum (1 in 10) which I have employed for the sedimentation test.

At first I used almost entirely Professor Wright's tubes, made according to his directions by Mr Dean of Hatton Garden, London. I have recently discarded these and employed a modification of my own, as I find Professor Wright's tubes extremely hard to handle, principally for two reasons.

a. The calibre of the stem of the tubes being too large, the fluid runs out at the point, even when compression is applied
to the indiarubber tube through which suction is made. Therefore it is extremely hard to keep the diluted serum in position while one is attempting to draw up an equal quantity of typhoid emulsion.

b. The mixing chamber, being symmetrical in shape and small in size, great care has to be taken, while drawing the fluid into this chamber, as it easily passes on to the thistle-shaped expansion, and is there absorbed by the cotton-wool plug, which serves as a barrier to the fluid passing into one's mouth.

The modification I have adopted is as follows:—

Two U-shaped tubes are joined together by their upright limbs as indicated in the diagram. The calibre of the tube is 1/16th of an inch. One extremity of each tube opens out into a "Thistle-shaped" expansion. Each opening can be plugged with cotton-wool and the whole instrument sterilised. When it is required to use the instrument, the cotton-wool plug is removed from one "thistle-shaped" expansion. A fixed quantity of the diluted serum is
Photograph of Sero-Sedimentation Tube of my own pattern.

Showing two U-shaped tubes joined at the top and bottom by solid glass rods.

The limb of the one tube is graduated as follows:

0.2, 0.4, 0.6, 0.8, 1 c.c.
then taken and allowed to flow into the tube by means of a fine glass pipette. To this is added 2, 3 or 4 times as much typhoid emulsion, according to the ultimate dilution required. The cotton wool plug is then replaced.

If a serum is at hand which has been obtained from a patient who is suffering from some disease other than enteric it can be placed in the other U-shaped tube and can act as a control on the serum which is to be examined.
The Reaction of Typhoid Serum on the

Eberth Bacillus.

Having studied the various methods of performing the test we can now look more closely into the reaction as seen

I. Microscopically
II. Macroscopically.

I. Microscopically.

The methods that come under this head are Widal's, McTaggart's, Delépine's, Grünbaum's and Muir's modification of Grünbaum's.

A slide preparation having been made, the following changes may be noted under the microscope.

If the serum be powerfully acting, almost immediately bacilli will be seen aggregated together into small bundles or clumps. Free motile bacilli are present in the field, but on carefully watching these it will be noticed that their movements, which were at first extremely active, become sluggish, and ultimately cannot be distinguished from Brownian
Diagrams representing the Reaction.

1. Reaction occurring.

2. Reaction occurring.

3. Reaction incomplete.

4. Reaction complete.

5. Reaction complete.

6. Reticulate formation of Clumps.
movement. If these free and motile bacilli come in contact with one of their neighbours or with one of the clumps already formed, it is found that they become adherent. If the clumps come into contact with each other it is also found that they adhere to each other.

The impression conveyed by watching the reaction occurring is that the surfaces, or cell envelopes of the bacilli become sticky and that if two bacilli come in contact they become glued together. It is also curious to notice the exertions put forth by one bacillus when it has become glued to a neighbour just as if it were trying to free itself.

This reaction goes on occurring until all the bacilli are agglomerated into these masses, and the intervening spaces, between the clumps, become quite clear. The motility of the bacilli also ultimately ceases.

At the commencement of an observation, clumps may be seen to move about, this is no doubt due to some active bacilli which have become adherent. The following phenomena constitute a complete reaction.

1. The formation of clumps of bacilli
2. The absence of all motility and abolition of Brownian movement.
3. The spaces between the clumps becoming absolutely free of individual bacilli.
If a serum be not powerfully acting, or if the dilution be too great, then, generally an incomplete reaction occurs. This consists of clump formation, but scattered over the intervening spaces motile or non-motile bacilli can be distinguished. In these cases the agglomerations are generally of small size and are slow in forming.

I have been able to verify the following statements made by Professor Delépine in one of his articles:— That red blood corpuscles do not exert any influence on the phenomena of agglomeration; and that bacilli are not attracted or repelled by them. I have never found a red blood corpuscle included in a clump.

In incomplete reactions the motility of bacilli is always considerably reduced, they however may become more active again, as will be noted, if the preparation be preserved for some hours.

Regarding the clumps in a complete or incomplete reaction, I may mention various forms I have seen in the observations made by myself.

A. Small or large clumps of bacilli loosely aggregated together; these are generally found in incomplete reactions.

B. Densely packed masses of bacilli; generally present in complete reactions.

C. Clumps of a reticulate appearance — which are generally of small size and
Microphotographs of the Reaction.
(The specimens were stained with Thionin blue.)

1.

No reaction.
 x 700.

2.

Reaction occurring.
 x 300.
3.

Reaction almost complete.
  x 700.

4.

Complete Clumping.
  x 300.
5.

Complete Clumping.

x 300.

6.

Complete Clumping.

x 300.
7. Reticulate formation of Clumps. x 300.

8. Reticulate formation of Clumps. x 300.
have 'arms' stretching out that join with those of neighbouring clumps. There may be one or more of these reticulate masses in a single specimen.

I have only seen this form twice and they both occurred in powerfully acting sera, where the reaction was complete. The 'arms' that I have just spoken of, consist of bacilli adherent to each other by their extremities, thus forming, as it were, chains, which may be thickened by having others adherent along their whole length. The clumps themselves in both instances consisted of bacilli loosely aggregated together.

D. Agglomerations of bacilli - in which the clumps appear made up of the ordinary rod-shaped bacilli with the addition of coccus-like bodies.

At first I thought that these coccus-like bodies might be the ends of rods whose long axes were parallel to the line of vision. This however is not always the explanation and I believe it to be due to the action of the serum on the bacilli. This is similar to the observations already made by Pfeiffer, of the action of cholera serum on the cholera vibrio (see Pfeiffer's Phenomenon - Introduction).

E. Clumps made up of granular and indistinct forms of bacilli. No true rods are distinguishable.

These last two forms - D and E - always
occur in a powerfully acting serum where the reaction is complete.

Fison points out that in pseudo-clumping, the organisms lie very much in the same plane, while in true clumping they are made up of organisms in a heap and are therefore not all in focus at the same time.

Sometimes in very weakly acting sera I have seen bacilli detach themselves from various clumps and begin again to swim about in the medium.

The reaction on Eberth's bacillus, which is sometimes met with when using sera from diseases other than typhoid, is always incomplete, active bacilli are always present in the field and the clumps are usually small in size and are often freely motile.

II. Macroscopically.

The methods that come under this head are all sedimentary reactions. Professor Wright's sedimentation tubes may be employed or my modification of his method. Ordinary test tubes of small calibre can also be used - this was first suggested by Durham as a convenient way of performing the test.

A typhoid serum having been obtained an emulsion of Eberth's bacillus in bouillon is added
and the required dilution made. The tube is then placed in an incubator for twenty four hours after which time the following changes are to be noted:

A. A whitish floccular precipitate at the bottom of the tube and the supernatant fluid perfectly clear.

B. The same as the last only the supernatant fluid not perfectly clear owing to the fact that all the bacilli have not been precipitated.

C. No floccular precipitate, and the whole column of the diluted serum homogeneously turbid.

The first class constitutes a complete reaction, the second an incomplete, and the third no reaction at all.

Sometimes at the end of twenty four hours, at the bottom of the tube, a fine granular precipitate may be noticed instead of being floccular, with the supernatant fluid turbid. This should not be mistaken for a partial reaction, but is due to the fact that dead bacilli are present in the fluid and they have fallen to the bottom owing to gravity.

If dead bacilli are employed for the sedimentation test, as advocated by Professor Wright, a floccular precipitate will occur in the presence of a typhoid serum just as when live bacilli are
used; but one always has the granular precipitate as well, and sometimes in an incomplete reaction the true clumps will be completely covered by the gravitation of dead bacilli, thus obliterating the result.

Professor Wright, himself, in one of his articles points out the necessity of not mistaking these two forms of precipitate when employing dead bacilli, and I myself think it is far better to use an active culture, so as to eliminate as far as possible any fallacies which may be introduced.

I have already pointed out some objections to using sedimentation tubes of the pattern recommended by Professor Wright, but I may here point out another very important one:—The calibre of the stem of the tube being very small, it is often impossible to note partial reactions in such a thin column of fluid, which if it were thicker would appear distinctly turbid instead of clear.

For this reason I was led to modify his tube and use one having a calibre of 1/16 inch or even greater.
Dilution of the Serum.

Throughout my observations I have almost constantly employed a dilution of 1 in 30, so that the results obtained should be more uniform.

In second or third observations I have sometimes altered the proportions.

The method I have employed for obtaining the necessary dilution is as follows:-

Having made a capillary pipette (by drawing out a piece of glass tubing 1/16 inch calibre after heating in a Bunsen flame), I place a mark on the stem at a convenient distance from the point. Draw up the 1 in 10 diluted serum from the U-shaped capillary tube as far as the mark and with care blow this quantity out on to the cell slide. I repeat this manoeuvre twice more, only on these occasions drawing up into the capillary pipette the typhoid emulsion, the preparation of which has been described already (See Dr Muir's Method).

Once these capillary pipettes have been used for an observation, I destroy them so that there is no chance of one serum becoming mixed with another.

From the above it will be seen that one
part of the 1 in 10 diluted serum has been mixed with two parts of typhoid emulsion, thus giving us an ultimate dilution of 1 in 30.

Widal, Delépine, Fison, and others have employed a dilution of 1 in 10, but I believe it is for this reason that observers have found agglomeration of the bacillus typhosus to occur in presence of sera taken from either healthy subjects or from those suffering from some disease other than typhoid.

Grünbaum in an article in the Lancet of 1896 states that out of 37 cases examined, the sera of 28 had an action on the typhoid bacillus. Of the 37 cases, 5 had had enteric, and 8 were suffering from it. The cholera bacillus was acted on in 16 cases out of 29 and the coli bacillus acted on in all 3 cases examined.

He has found the reaction present in some of the following diseases, Chronic Rheumatism, Jaundice, Carcinoma, Tetanus, etc., and specially refers to the serum of Jaundice being capable, when undiluted, of producing an action as rapid and as complete as an enteric serum. I cannot agree with him on this point as the two cases of Jaundice that I have examined have both given a negative reaction.

Grünbaum, in a more recent paper, makes the
following statements which I think are of great importance: "I have not yet come across a case of enteric in which the serum would not react at some time in the course of the disease in the dilution of 1 to 32. . . . On the other hand I have not yet met with a serum other than that of a typhoid patient, which would react in this dilution."

Some observers have recorded cases where they have obtained positive results in diseases other than enteric with a dilution as high as 1 in 30, but they have, it appears to me, forgotten to exclude the possibility of a previous attack.

Those who work with a dilution of 1 in 10 hold that they are able to obtain the reaction at an earlier date in the course of the disease than those who work with a higher dilution of 1 in 30. This may certainly be the case, but surely it is better to defer a difficult diagnosis a day or two instead of introducing a possible error by working with a dilution of 1 in 10.

Grünbaum has also pointed out that there are certain cases of enteric where the reaction appears more rapidly and is more complete when a dilution of about 1 in 30 is employed than when a dilution of 1 in 10 is used.
If as Gruber and Durham state, the agglutinins present in the blood of an enteric patient are in direct proportion to the state of immunity and that these agglutinins are used up either by chemical combination or by actual destruction during the process of agglomeration that occurs when the bacilli are brought into contact with such a serum; then the question of dilution is of the utmost importance and very materially affects the question of the time limit necessary for the reaction.

It is also of importance from another standpoint; for instance if one dilutes an enteric serum with the emulsion of typhoid, it is conceivable that one might add a larger proportion of bacilli than the amount of agglutinins present. That these agglutinins would only be able to destroy or at least to act on the cell wall of a certain number of bacilli as they are themselves used up in the process and that therefore an incomplete reaction would be obtained.

At the present state of our knowledge the question of the dilution of the serum will have to remain unsettled. When observers like Widal, Delépine and Fison, who have always worked with a dilution of 1 in 10, have put on record a large num-


ber of cases, other than enteric, where no reaction was obtained with the serum; we are at a loss to know why some sera in a concentrated form give the reaction while others do not.

There is a theory that normally in every person agglutinins are present in the serum, and of course certain individuals may possess the power of producing a greater quantity than others.

One is able to estimate roughly the intensity of reaction of a given typhoid serum, by observing to what degree it is capable of being diluted without losing its agglutinative power. Here again, however, so much depends on the culture one is employing that the observations on this point are almost valueless. A given serum with one culture may be diluted up to 1 in 500 and still the reaction be marked, while with another which is more attenuated the dilution may only be carried to 1 in 100 without destroying the agglutination.
Varied opinions are still held concerning the most suitable time limit which should be employed. Some observers always use a time limit of \(\frac{1}{2}\) hour, others 1 hour, and others again 2, 3 or 4 hours.

Naturally the whole question primarily turns upon the dilution of the serum that one uses. It is obvious that if we are to employ a time limit in our observations, it should be comparatively shorter when the serum is concentrated than when it is highly diluted. Grünbaum has however shown that one serum he examined gave the reaction quicker in a dilution of 1 in 30 than 1 in 10.

If one employs a constant dilution of 1 in 10 for all observations, then I think \(\frac{1}{2}\) hour is an ample time limit; that is to say if the reaction does not occur within this period then it will not occur at all.

For a dilution of 1 in 30 I hold that a time limit of \(\frac{3}{4}\) hour is plenty. It will however be noted that in my record of cases where the serum has been examined in diseases other than typhoid that I have
often carried on my observations for two or three hours. The reason for this procedure was simply to try and ascertain if a serum other than typhoid was capable of exerting any influence on the Eberth bacillus after prolonged contact.

Many observers, using a dilution of 1 in 30, have pointed out that often they have been able to obtain a reaction after two or three hours when they have failed with using a shorter period. I cannot agree with this as I have never yet found a typhoid serum which, when diluted thirty times, was incapable of producing a reaction within the hour. I have however found that when one employes an attenuated culture the reaction is much slower in appearing than when an active virulent culture is used, and is also never so complete.

I believe that all the results put on record which go to prove the fallibility of the test, are solely due to improper cultures being used. I do not think too much importance can be laid upon the necessity of using active virulent cultures. More attention should be paid to the culture than to any other part of the test. Fison, who works with a dilution of 1 in 10, has pointed out that in one case a typical reaction was obtained after an hour
and a half; death of the patient occurred and no enteric lesion could be found at the necropsy. He and Grünbaum also state that they have found many diseases other than typhoid giving the reaction after a period of twenty four hours. As however Grünbaum in the next paragraph says that he thinks it advisable to use an attenuated culture I do not lay much stress on the importance of his observation.

I have never yet met a serum (which was certainly not an enteric serum) that gave a reaction after a period of twenty four hours in the presence of bacilli taken from an active virulent culture. On the other hand I have found incomplete reactions occurring when an attenuated culture was used.

If a reaction occurred so constantly as observers make out when a lengthy time limit is employed, then the sedimentation test would be of little value as here one always has to lay aside the tube, and an observation cannot be recorded for at least twenty four hours.

In my record of cases diagnosed as enteric fever, I have never found it necessary to carry an examination of a serum further than one hour.
Date of Appearance of the Reaction.

I have not been able to make many observations on this point myself as the majority of patients admitted into hospital are generally at the end of the first or beginning of the second week of the disease.

Many observers have however published statistics and have shown that the agglutinative action may be looked for as early as the 3rd, 4th, and 5th days, some even have found it on the 1st and 2nd. I will here give a table, taken from the British Medical Journal, of cases quoted by Drs. Wilson and Wesbrook at the British Medical Association of last year.

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These tables are of little value as it is exceedingly hard to state definitely that a patient
is in such and such a day of the disease.

It has been pointed out that the reaction may be found present on one day and absent on the next. One case of this description came under my notice. The reaction was found to be positive on the 14th day of the disease, absent on the 15th and present again on the 17th. Drs Wilson and Wesbrook have also published a case where the reaction was absent on the 9th day, present on the 12th, absent on the 13th, present on the 19th and 20th, absent on the 21st and present on the 25th day of the disease.

The reason of this appearance and disappearance of the agglutinative action of a serum is unknown, but it might be due to an alteration in the amount of fluids in the blood on different days.

I have in the report on cases, given a table of the days of disease on which I obtained a positive reaction. I think it is generally acknowledged that the reaction is, certainly in a case of enteric fever, present by the 7th day of the disease.
A Note on the Typhoid Culture
and the Reaction of Typhoid
Serum on the Bacillus Coli
Communis.

From the work of recent observers it has been
shown that organisms allied to the Eberth bacillus
give a positive reaction with typhoid serum.

Landsteiner, Durham, Peeham, Courmont, Ben-
saude and Christophers are a few of the chief obser-
vers who have worked at this subject.

Durham divides the Eberth-like bacilli into
three groups.

1. The Eberth group - or the true typhoid
bacillus.

2. The Gärnter group.

3. The Escherich group - or the true bacillus
coli.

The two latter have been shown to give the
Widal reaction in the presence of a typhoid serum.

I propose here to give the main features of
difference in the mode of growth etc., between group
1 and group 3.

Group 2 is exceedingly closely allied to the
ture Eberth bacillus and Durham states that this
group of bacilli have a strong resemblance to certain
organisms he has been able to separate out from cases of meat poisoning.

<table>
<thead>
<tr>
<th>Eberth Group</th>
<th>Escherich Group</th>
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<tbody>
<tr>
<td>1. On acid potato gelatine.</td>
<td>Growth slow and colonies small.</td>
</tr>
<tr>
<td>4. Nutrient litmus solution.</td>
<td>Slight acid reaction.</td>
</tr>
<tr>
<td>5. Flagella.</td>
<td>About 10</td>
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Landsteiner was the first to point out that the Gärtner bacillus gives a positive reaction with typhoid serum, and Durham has since been able to confirm this observation during the recent epidemics at Maidstone, Clifton and King's Lynn.

A full account of his work concerning the reaction typhoid serum has on this organism is to be found in the Lancet, January 15th, of this year.

I have not been able to test this bacillus with the sera taken from typhoid subjects that I have examined as I have not been able to procure a typical culture.
What has interested me far more is the work of the other observers, whom I have mentioned above, as they all, with one accord, state that the \textit{bacillus coli communis} gives a positive reaction in the presence of typhoid serum. If this be the case then the Widal reaction will be of little value for diagnosing such cases where the \textit{bacillus coli} is the organism giving rise to obscure symptoms which have a resemblance to those of enteric fever.

My observations on this point correspond with those of Durham and others, namely that the \textit{bacillus coli} does not give the positive reaction, viz: clump formation, etc., in the presence of typhoid serum when it has been sufficiently diluted. On the other hand I must say that when working with a dilution of $1/2$ or even $1/15$ I have noticed that sometimes the motility of the bacilli appears to diminish and clumps begin to form; the reaction, however, is never complete (as when a typhoid culture is used) even if the specimen be examined two to three hours after the commencement of the observation. The clumps only consist of an aggregation of two or three bacilli which on careful watching are found to possess the power of disengaging themselves and begin swimming about again in the medium.
Christopher points out that normal human serum gives a positive reaction with the bacillus coli, and he believes that the serum of a typhoid subject has two distinct actions, 1. "On the bacillus typhosus due to infection by this organism." 2. "On the bacillus coli, an action which is present alike in typhoid and non-typhoid sera." It has occurred to me that the reaction produced by the action of a typhoid serum on the bacillus coli, which some observers have noted may be due to the following reason.

As is well known the bacillus coli exists normally in the small and large intestine of man and that it increases enormously in numbers when any pathological condition arises - such as typhoid fever. It has been pointed out that at times it enters the lymph channels, especially the appendix and bile ducts, giving rise to suppuration. Now, if this be the case surely we may infer that absorption of these bacilli or their products takes place through the inflamed or may be ulcerated Peyer's patches in enteric fever. If, therefore, absorption occurs in small and repeated doses what more do we require for a state of immunity becoming established in such a subject against this particular bacillus in addition
to the Eberth bacillus?

From the experiments of Pfeiffer and others, we have seen that immunity can be produced in animals by repeated inoculations of various organisms.

In the case of a typhoid subject we have much the same thing taking place, and I hold that such a subject is immunising himself against the Eberth bacillus as well as the coli bacillus.

For this reason, therefore, we would naturally expect a typhoid serum to give a positive reaction with both these organisms.

Christophers in his paper, as I have already said, shows that normal human serum gives a positive reaction with the bacillus coli and I account for this in the same way. These organisms are constantly passing into the lymph and blood channels and therefore immunity is being established which can be demonstrated by the action of the serum on these organisms, or I should say other observers can demonstrate this action.

This theory of double immunity which I have put forward ought to exist in all cases of typhoid fever if it is correct. However, from practical experience I have been able to prove that this is not the case. Many of the typhoid sera which have
had a marked and rapid action on the Eberth bacillus
have failed to give any reaction with the bacillus
coli communis.

Dealing with this question of the reaction
of the coli bacillus in the presence of a typhoid
serum, the following experiment occurred to me as
being one which would be interesting to perform and
might perhaps throw some light on the matter.

If with a powerfully acting typhoid serum
it is possible to obtain a reaction with the bacillus
coli as well as with the Eberth bacillus, one ought
not to be able to obtain a culture of the coli after
the reaction has occurred. In the case of the ac-
tion of typhoid sera on Eberth bacilli it has been
pointed out that death of the bacilli, as well as
agglomeration, occurs, and therefore no growth is
obtainable after such a reaction. This proves
that the sera of such patients has a double action
on these bacilli.

1. A toxic action, causing the death of the bacilli
2. An agglomerating, producing clump formation.

Now, if a typhoid serum has an agglomerating
action on the coli bacillus, does it also have a
toxic action? This led me to perform the following
experiment:—
Note on the action of Typhoid Serum on the Bacillus Coli communis.

Donald H. Hutchinson, M.D.
The common member of the bacillus coli communis normally present in the bowel might very readily induces in the animal a condition of immunity against their product of these and this might be accompanied by an agglutinating action. Such an influence would naturally seem to make more manifest when the bowel lining membrane of the bowel was in such pathological condition as it is in typhoid fever, and the double agglutinating action of the serum for such cases might be thus influenced.

Whether typhoid serum has the same toxic action in the bacillus coli communis that it has in Shwartzbacilus has not so far as have been

The following experiments were undertaken for the investigation of this question.
Having obtained a powerfully acting typhoid serum, add to it a mixed emulsion of typhoid as well as coli bacilli, and after waiting for the reaction to be complete on the former, see if it was possible to make a culture of the latter.

In detail my method of procedure was as follows:-

1. Draw up with all antiseptic precautions about 1 c.c. of blood into a sterile blood capsule (something of the same kind as that recommended by Professor Delepine, see page 30.)

2. Hermetically seal the two ends and allow the serum to separate.

3. Decant the serum by means of a glass pipette into a sterile U-shaped tube, with a thistle-shaped expansion at each extremity so that it can be easily plugged with cotton wool to prevent contamination.

4. Add to this serum an emulsion of typhoid and coli bacilli. Care must be taken that too great a number of bacilli are not added so that the reaction may be as complete as possible.

5. Plug the two ends of the U-shaped tube with sterile cotton wool and place in an incubator for 24 hours so as to allow sedimentation to occur.

6. Remove the tube from the incubator after this
time has elapsed and inoculate an agar agar tube with one drop of the fluid. If a growth occurs examine it to see if it consists of coli bacilli or coli and typhoid bacilli. If the serum has been a powerfully acting one all the typhoid bacilli should have formed into clumps, sunk to the bottom of the U-shaped tube and no growth of these organisms should be obtained. On the other hand, if the typhoid serum has no action, or I should say no toxic action on the coli bacillus, then an abundant growth should be obtained of these organisms which can be demonstrated by the tests given a few pages back.
Result of Experiments.

Experiment I.
Performed with my own blood, which at present possesses a marked agglutinative action on the Eberth bacilli.

I produced this effect myself by inoculation following the lines laid down by Professor Wright.

As I shall be dealing with this question of acquired immunity at a later period I shall not enter into it here.

Having carefully made aseptic a portion of skin around the root of the thumb nail, two or three rapid stabs were made with a sharp needle and about 1 c.c. of blood was drawn up into a sterile blood capsule. Both ends of the capsule were then sealed in a flame, great care being taken not to allow the contents to become heated. This was then placed aside for 24 hours, and the serum allowed to separate.

One end of the capsule was now broken off with sterile forceps and a capillary pipette inserted. The serum was drawn off and decanted into one of my U-shaped sedimentation tubes.

To the serum was added equal quantities of emulsions of typhoid and coli bacilli which had been
grown in broth for 24 hours. The dilution of the serum, after the emulsion was added, was 1 in 20. The "thistle expansion" was plugged with cotton wool and sedimentation allowed to take place for 24 hours. After this period an agar agar tube was inoculated with a platinum loopful of the diluted serum. The next day an abundant growth was noticed and the question I now had to deal with was what did the growth consist of? Was it a pure coli culture or a mixture of coli and typhoid? Various tests were applied:

A. Neutral litmus solution in gelatine was rapidly made acid.

B. Rapid production of gas in nutrient gelatine to which lactose had been added.

C. Milk at 37°C clotting in from 18 to 24 hrs

D. No agglomeration of the bacilli was produced when mixed with a powerfully acting typhoid serum in a dilution of 1 in 20 and a time limit of two hours.

I took the precaution of testing this typhoid serum at the same time, with the original Eberth culture, or I should say a culture obtained from the original one that was employed in this experiment and marked agglomeration resulted in ½ hour.

The Eberth culture that was used for all
these experiments was also tested and it was found that

1. Milk was not clotted.

2. Neutral litmus gelatine did not become acid after 48 hours incubation, and even after 14 days the acid reaction was very slightly marked.

3. No production of gas in lactose gelatine.

A coloured photograph of the litmus reaction is shown on the accompanying page. Tube No. 1. was inoculated with the Eberth culture used in the experiment. Tube No. 2. was inoculated with the growth obtained from the serum.

Although to my mind it was now clearly proved that the culture obtained from the serum was a pure coli growth and did not contain any typhoid bacilli, yet I thought it advisable to apply still further tests, and the two I adopted were those first recommended by Dr Hiss for the differentiation of the coli and typhoid bacilli.

He uses two different media, one for tube cultures and the other for plate cultures. They consist of the following ingredients.

**Tube medium.**

- Agar agar 5 grms.
- Gelatine 80 "
- Sodium Chloride 5 "
- Extract of beef (Liebig's) 5 "
- Glucose 10 "

to one litre of water and the
whole should have a reaction indicating 1.5% of normal acid. Phenolphthalein being the indicator.
The typhoid bacillus should produce a uniform clouding in this medium at 37°C. in eighteen hours.

**Plate medium**

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<tr>
<th>Ingredient</th>
<th>Quantity</th>
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<tr>
<td>Agar agar</td>
<td>10 grms.</td>
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<tr>
<td>Gelatine</td>
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<tr>
<td>Sodium Chloride</td>
<td>5 &quot;</td>
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<tr>
<td>Extract of beef (Liebig's)</td>
<td>5 &quot;</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 &quot;</td>
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...to one litre of water and the whole should have a reaction of 2% normal acid. Phenolphthalein being the indicator. The typhoid bacillus produces in this medium growths, which are small in size and have fringing threads and outgrowths around their margins.

(See British Medical Journal. Dec. 18th. 1897.)

Having prepared these media I now tested the culture that I had obtained from the serum, by inoculating a tube and a plate. The tube medium, after twenty four hours incubation became distinctly clearer, owing to acid production, small bubbles of gas were formed, and the growth itself was irregular and in masses. The colonies on the plate medium were thick and did not show the characteristic "fringing" of a typhoid colony described by Dr Hiss. After 24 hours incubation they were the size of about a pin's
head. All the growths on the plate were identical. I was now certain that I was dealing with a pure coli growth and not a mixture of coli and typhoid bacilli. By this experiment it has been shown that a serum of an immunised subject such as I am, has a marked action on the Eberth bacillus and none on the bacillus coli communis.

This experiment with my serum is still more interesting since I have recently been suffering from appendicitis and ought by rights to have shown an action on this organism as the above disease is often supposed to have its origin in infection from the bacillus coli.

Experiment II.
Performed in exactly a similar manner to experiment I, only the serum used was obtained from a patient suffering from enteric fever. The result was no less convincing, a growth being obtained from the serum which had had a mixed emulsion added to it and had been incubated for 24 hours.

The above tests were again applied and so I have been led to believe that again a pure coli growth was obtained, while all the typhoid bacilli had settled at the bottom of the tube in the form of clumps.

The growth gave no reaction in the presence
I. Inoculated with the culture of Bacillus Typhosus used in experiments Nos. 1, 2, 3. Showing no gas formation and no acid reaction.

II. & III. Inoculated with growth obtained from serum in experiments 1 and 2. Showing gas formation and acid reaction.
of a typhoid serum.

**Experiment III.**
Performed with serum obtained from a healthy subject.
To it was added a mixed emulsion of typhoid and coli bacilli. Having been incubated for 24 hours an agar agar tube was inoculated with a portion. A marked growth was obtained, which gave all the ordinary reactions of the bacillus coli.

In the presence of a powerfully acting typhoid serum, however, an incomplete reaction was obtained, and when a Petri dish of Dr Hiss' culture medium was used two distinct forms of colonies could be distinguished.

A photograph will be seen on the next page of the Petri dish, showing the two forms of growth.

This experiment with a normal serum acted as a control on the others, because if I had not been able to obtain two forms of growth on a Petri dish it would have shown that my methods of working had been deficient.

**Experiment IV.**
An ordinary enteric serum was taken and to it I added a simple emulsion of typhoid bacilli.

After allowing sedimentation to occur for
Growth obtained from enteric serum after inoculation with a mixed emulsion of typhoid & coli bacilli, showing small round growths clear & well defined. These gave all the reactions of the coli bacillus.

Growth obtained from enteric serum after inoculation with a simple emulsion of typhoid bacilli, showing growths which are diffuse with the margins ill defined.

Growth obtained from a healthy serum after inoculation with a mixed emulsion. Two forms of growth are seen: a. Those giving the coli reactions.
   b. " " typhoid "
The plate medium below the red line is cracked.
24 hours I inoculated an agar tube with a drop of the supernatant fluid. A marked growth was obtained which turned out naturally to be a growth of typhoid bacilli.

This experiment is apt to throw some doubt on the value of the preceding ones, but I think the following is a sufficient explanation.

First the serum I was using was not nearly as powerful as those used for the other experiments; and secondly I noticed that all the clumps had not sunk to the bottom of the tube, but some were adherent to the sides, and therefore for this reason the agar tube in all probability had been inoculated with a portion of one of the clumps.

Experiment V.
This consisted of performing the test over again, only using a very powerful typhoid serum. No growth was obtained from this case after sedimentation had been going on for 24 hours. When I found this action was complete I then added an emulsion of coli bacilli and set the tube aside for another 24 hours. After this time, having inoculated an agar tube, I was able to obtain an abundant growth which proved to be the coli bacillus.
From these experiments I think I have clearly shown that an enteric serum has no action on the bacillus coli communis; and I think the observations made by Pecham and others, that an enteric serum has an action on the coli bacillus as well as the Eberth is due to the fact that impure cultures were employed.

Let us now turn our attention more fully to the bacillus typhosus.

The following are points which should be carefully attended to.

1. The most suitable media for the growth of this bacillus is either neutral bouillon or slanting tubes of neutral nutrient agar agar.

2. No culture should be employed which is more than 24 hours old. If dead bacilli are used they should be obtained from a culture of about this age. If other cultures are used then groups of dead bacilli may be seen under the field of the microscope. These may easily be mistaken for clumps formed by the action of the serum.

3. On examining a drop of emulsion prepared from an active culture, under the microscope the bacilli should be freely motile, no Brownian movement should be present, and the bacilli should not show any tendency to adhere to each other.

4. Care must be taken that the culture em-
ployed is not attenuated, or agglomeration will occur in the presence of sera taken from subjects who are healthy or who are suffering from diseases other than typhoid. Professor Delépine, Dr Fison and other others have all pointed out the importance of this point.

The most suitable method of keeping ones culture active and virulent is as follows.

A. Make a stab culture in gelatine and allow it to grow at the ordinary room temperature.

B. From this culture inoculate agar agar tubes from day to day as required.

Of course as is well known all cultures grown for some time outside the body lose their virulence, also that the best method of increasing the virulence of any particular organism is to cultivate it from animal to animal, or to inject it into an animal along with another organism.

Professor Delépine gives the following method of keeping a culture virulent. Starting with a growth on some solid medium, inoculations may be made from this in broth cultures from time to time as required.

Grünbaum makes the following statement in one of his articles. "It is desirable to use an attenuat-
ed culture of the typhoid bacilli, since it seems to be less often affected by normal sera." This does not correspond with what I have just said, as many have shown that agglomeration is more likely to occur with an attenuated than with a virulent culture, in the presence of various sera employed. There seems to be an undoubted relation between the virulence of the organism and the agglomeration.

I have noted that when using a culture which has become contaminated with some other organism, that clump formation occurs in the presence of a typhoid serum. The reaction is however incomplete and a clear zone can be seen around each clump which is free of all bacilli. It looks exactly as if the clumps had some substance enveloping them, which prevented the foreign organism from coming in contact with them.
The Question of Immunity and Vaccination against Typhoid Fever.

From the experiments of Pfeiffer, Kolle, Metchnikoff and others, described somewhat fully in the Introduction, it will be noted that these observers were able to establish a state of immunity, artificially, in animals. That is to say, an animal receiving repeated inoculations of small doses of a particular virus is able ultimately to overcome the ill effects that a large dose would have had, had it been injected in the first instance.

For example if one takes a guinea pig of about 500 grammes and inoculates it at intervals with about 1/3 of a culture of dead typhoid bacilli, one is able after a certain time to inject 1/2, 1, or 2 platinum loopfuls (a loopful weighing about 2 m. grammes) of an active virulent culture without death of the animal resulting. Whereas if such doses of virulent culture had been given in the first instance, death would almost certainly have resulted within a few hours.

The reason why an immunised animal is thus
able to resist the toxic effects that such large doses would have had on a normal animal is we believe due to the fact that substances have been produced in the blood which are capable of destroying or antagonising those substances which produce these toxic effects. What these antagonistic substances are I will deal with at a later period. At present let us turn our attention to the subject of the production of artificial immunity against typhoid fever or in other words vaccination against typhoid fever in the human subject.

Professor Wright and Surgeon Major Semple in an article in the British Medical Journal, January 30th 1897, described a method of vaccination for the human subject which had been suggested to them sometime previously by Mr Haffkine, who had been performing vaccination against cholera.

Pfeiffer and Kolle have at the suggestion of Professor Wright been performing typhoid vaccinations in exactly similar a method as adopted by him. He however primarily obtained the method from Mr Haffkine's article on anticholera inoculations.

Professor Wright points out that in the process of vaccination two objects are aimed at.

Firstly to produce a certain degree of immunity which shall be somewhat similar to that
obtained from an actual attack of the disease; and secondly to produce a degree of immunity which shall have no risk to life.

The best method of producing immunity in a person is by injecting organisms or their products, or both, into the system. The method of obtaining the second object may be performed by various methods, but that which concerns us most is that described in the above mentioned article by Professor Wright — namely the injection of dead bacilli and their products into the system.

The method employed by them for preparing anti-typhoid vaccine is as follows:

To a twenty four hours culture of typhoid bacilli a certain fixed quantity of bouillon is added. An emulsion having been made, it is drawn up into a number of calibrated pipettes. These are hermetically sealed and subjected to a temperature of 60°C. for 5 or 10 minutes.

To test if the bacilli have been killed by exposing them to this temperature allow the contents of one tube to flow over the surface of an agar agar tube. Incubate it for forty eight hours and see if any growth is obtained.

If we add eight cubic centimetres of bouillon to a typhoid culture, and take eight equally
calibrated pipettes, drawing off an eighth part of the emulsion into each and inject one of these into a subject — that subject is said to have had an injection of 1/8 of a culture. It appeared to me when preparing vaccine tubes for myself that the above method had certain deficiencies which are as follows:—

That we may obtain a far more rapid growth of bacilli in one twenty four hours than in another, therefore a subject getting an eighth of a culture as an injection would in one case receive far more bacilli than where the growth of the bacilli had been slow.

Anyone working with cultures cannot fail to note how at times abundant and strong growths occur in twenty four hours while at others they are small and feeble.

Of course we have no method at our disposal of inoculating our agar tubes with always the same number of bacilli and for this reason alone a more abundant culture may be obtained in one tube than in another. Again, the media that we are using in our tubes may contain a different quality or quantity of bouillon in each tube, therefore the bacilli may have a more suitable medium for their growth in
one tube than in another.

Professor Wright gets over these difficulties by using a typhoid culture of such a strength that 1/4 tube of a twenty four hours culture constitutes a lethal dose for a guinea pig of 350 to 400 grammes when inoculated.

The dose that may be employed of these vaccines, for the human subject, may range from 1/20 to 1/4 of a culture.

I must here take the opportunity of thanking Professor Wright for the kindness he has shown me in supplying me with information that I required, and also a quantity of typhoid vaccine tubes prepared by himself.

Having read his article in the British Medical Journal and studied the results he obtained by vaccinating the human subject against typhoid fever, I determined to render myself immune when a suitable opportunity arose. For this reason I entered into communication with him, and he spared no pains to advise me in the procedure I should adopt.

I will now continue with his own observations regarding vaccination and will then give a full account of my own case.

On inoculating a human subject with 1/20 to 1/6 of a twenty four hours culture of the bacillus
typhosus, certain clinical symptoms present themselves which are as follows:

1. Local tenderness at seat of injection.
2. Feeling of chilliness commencing two or three hours after inoculation.
4. Restlessness at night.

All symptoms passing off in twenty four hours. Where a larger dose is given, say for instance 1/4 of a culture, severer symptoms arise.

1. Two or three hours after inoculation, great local tenderness increasing in severity and extending to the neighbouring glands. Greatest intensity about twelve hours after inoculation.

2. Patch of congestion around seat of inoculation, two or three inches in diameter.

3. Red lines, showing course of inflamed lymphatics.

4. Faintness and collapse.

5. Nausea, sometimes resulting in vomiting.


These symptoms, he found generally disappeared in about twenty four hours.

Professor Wright administered calcium
chloride in about 50 grain doses to those patients who received inoculations to prevent the possibility of a decrease of the blood coagulability or oedema arising.

The changes produced in the blood by such inoculations are similar to those seen in the blood of a subject suffering from enteric fever. (See "Note on Reaction." ) Undoubtedly sedimentation and agglomeration are the two chief features to be looked for.

Having thus, he says, inoculated such given quantities of typhoid vaccine into the blood, we are able by various successive dilutions of the blood, to note the agglomerating and sedimentary powers of the serum.

"The quantitative results which are obtained by this method of successive dilutions may be expressed in terms of 'sedimentation units' or 'preventive units.' Thus we shall be able to speak of a blood which has the power of producing sedimentation in a tenfold dilution as one which contains one sedimentation or preventive unit. And again, a blood showing sedimentation after being diluted one hundred times as one which possesses 10 sedimentation or preventive units."
There is little if any risk to life in carrying out these inoculations on the human subject. That is to say if the vaccine has been carefully prepared and subjected to a suitable temperature. Of course one is unable to tell absolutely definitely whether all the bacilli in the vaccine tubes have been killed. Some may survive the temperature of 60°C. Therefore everyone who has the operation performed does run a certain amount of risk of having live bacilli injected.

It has occurred to me that this risk might be entirely obviated by filtering the emulsion of dead bacilli through a Pasteur Chamberlain filter, but it would be first necessary to pound down the bacilli in a mortar, so as to liberate the toxines contained in the protoplasm within the cell enveloped.

Haffkine has now carried out nearly a hundred thousand anti-cholera inoculations without a single death, so the method may be considered safe if the vaccine is carefully prepared by an experienced hand.

The next question to be dealt with is, does an anti-typhoid vaccine produce a state of immunity sufficient to kill living bacteria.

It has been pointed out by Professor Wright and I have myself been able to verify his observation
that a blood exhibiting twenty sedimentation units fails to kill living typhoid bacilli. From this it appears that we cannot rely on the blood of a vaccinated person killing typhoid bacilli which may enter his system.

On the other hand, taking for granted that a vaccinated subject has a certain degree of immunity conferred upon him; (by the sedimentation observed in his blood) are we justified in assuming that this immunity is sufficient to in any way lessen or alter the severity of an attack of enteric faver? We have every reason to make this inference as will be seen by the following experiment which is simply a repetition of that performed by Pfeiffer. If a guinea pig be carefully inoculated by repeated small doses of dead typhoid bacilli, and shows a marked agglomerating power by the action of some of its serum on live bacilli, it is found to be extremely resistant to the effects that would be produced by an injection of a loopful of an active virulent culture into a non-vaccinated guinea pig.

I have been able to render guinea pigs so thoroughly immune against cholera and typhoid that they have been able to resist entirely the toxic effects of an injection of 1½ loopfuls of an active
virulent culture.

Professor Wright has also shown this to be the case in the human subject. On September 5th 1896 he inoculated one of his patients showing a sedimentary reaction of twenty units with 1/6 of a tube of living virulent typhoid bacilli. No evil results following. I have not considered it justifiable to perform this experiment on myself as I am not in a sufficiently good state of health, but instead, at intervals I have taken by the mouth minute doses of 1 drop of live bacilli in bouillon.

I will now proceed to relate in full my own case, with the results obtained regarding the production of agglutinins in my blood.

Twice before I received the inoculation I had my blood carefully examined, with the result that no agglomeration of bacilli occurred and no sedimentation was to be observed.

The inoculation was performed at the Royal College of Physicians Laboratory on the 8th October 1897 at 3.30 p.m. by Dr Noel Paton in the presence of Dr Gibson and Dr George Chiene.

1/8 of a culture in bouillon (which had been previously exposed to a temperature of 60°C. for ten minutes) was injected deeply into muscle substance
The seat of the injection was the left flank, a little above the posterior superior iliac spine.

All the ordinary antiseptic precautions were carried out as regards the preparation of the skin surface and hypodermic. An ordinary hypodermic was used. Immediately after the inoculation my temperature and pulse were taken, and this was carried on regularly every half hour till 1 a.m. on the 9th of October. After this a daily temperature chart was kept for the next ten days.

**Symptoms.**

October 8th. For 1½ hours after the injection no constitutional symptoms were to be observed. There was a slight pain on movement at the seat of the injection after 1 hour, which gradually began to increase in severity.

At 4.30 p.m. my temperature rose to 99.5°C.

5 " slight frontal headache. Temperature 99.6

5.30 " Headache severe; the pain having a sharp shooting character, commencing over the temporal bone on each side and meeting in the middle line in front. What struck me as remarkable was the
rapidity with which the intensity of the headache increased. At this time I began to suffer from slight giddiness, nausea and retching. My extremities had the feeling of intense cold, which proved to be really the case as told me by another observer. This last symptom lasted for $\frac{1}{2}$ hour. My temperature was now 99.8 and pulse 84.

6 p.m. I now began to experience the sensation of intense heat. The other symptoms continued as before. The temperature rose to 100.2 but pulse remained the same.

I kept walking about till 5.45 but eventually had to lie down.

Profuse perspiration had now set in, which was specially noticeable on the palms of both hands where large beads of sweat were to be seen.

From this time onwards the headache began steadily to diminish in severity and the nausea and retching had almost stopped. If however I rose from the prone position these symptoms, with giddiness returned. My temperature was now 100.4
and pulse 88.

8 p.m. No nausea or retching. Headache had disappeared and the perspiration was less. I was now able to walk about the room with greater comfort although the pain at the seat of injection was increasing in severity. Temperature 99.6. Pulse 80.

9 p.m. and onwards, absence of all constitutional symptoms. Temperature 99.2. Pulse 80. The temperature returned to normal at 12.30 p.m.

It will be seen at a glance by looking at the above chart the gradual rise and fall of the
temperature for nine hours after the injection. The chart is divided into two halves. That to the left being a half hour chart and that to the right a daily chart, commencing on October 9th.

During the whole of the night after the injection I was very restless and only obtained snatches of sleep. The pain in my back around the seat of the inoculation was now severe. The inguinal glands on the left side had begun to be enlarged and tender.

October 9th  The pain in my back and groin was very severe all day and prevented me from bending. The pain was that of an ordinary acute inflammation. In the afternoon the discomfort was so great, and being afraid of abscess formation I had a large 1 in 60 carbolic poultice applied over the inguinal glands and over seat of injection. This gave me great relief and I was able to move about more freely. My temperature that night at 8 p.m. was 99.2. There was an area of redness around seat of injection about four inches in diameter. Lymphatic vessels were inflamed and injected and could be traced running to the inguinal glands.

October 10th  The pain which troubled me so much on the previous day began gradually to pass off. My
blood was carefully tested and no reaction or agglomeration was obtained. The serum was diluted 1 in 10 and a culture of twelve hours growth was used. Time limit employed 1 hour.

I was restless again all night. My temperature rose from normal in the morning to 99.6 in the evening.

October 11th. Pain in groin and back almost absent, the glands however were still hard, enlarged and tender to the touch. All local symptoms had disappeared by October 14th, and I was once more in my usual health.

After the injection on the 8th an examination was made of a portion of the emulsion to see if any of the bacilli were alive. No growth was obtained on agar agar after 48 hours incubation. Active Brownian movement of the bacilli was to be observed under the microscope. A film preparation was also stained and mounted so as to see if the emulsion was a pure one of typhoid bacilli. This was found to be the case.
Photograph showing sedimentary power of my own serum after having been inoculated.

There is a control serum in the other tube showing even turbidity of the fluid.

Dilution of sera 1 in 50.
**Table showing Dates of Examination of Serum and the Results obtained.**

The methods employed were Dr Muir’s modification of Grünbaum’s method and the sedimentation method.

<table>
<thead>
<tr>
<th>Date of examination of serum</th>
<th>No. of observations</th>
<th>Method employed</th>
<th>Dilution of serum</th>
<th>Reaction</th>
<th>Time limit</th>
<th>Motility of bacilli.</th>
<th>Rapidity of agglomeration</th>
<th>Size of clumps</th>
<th>Age of culture</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>1897</td>
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<td>&quot; 12</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1:20</td>
<td>1</td>
<td>1&quot;</td>
<td>Partially destroyed</td>
<td>&quot;</td>
<td>Slow</td>
<td>Small</td>
<td>24 hr.</td>
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<td>2</td>
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<td>1:30</td>
<td>1/2</td>
<td>1/2 hr.</td>
<td>Absent</td>
<td>-</td>
<td>-</td>
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<tr>
<td>&quot; 14</td>
<td>&quot;</td>
<td>&amp; Sedimentation</td>
<td>1:30</td>
<td>&quot;</td>
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<td>&quot;</td>
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<td>&quot;</td>
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<td>24 hr.</td>
<td>24 hr.</td>
<td>Absent</td>
<td>Medium</td>
<td>Medium</td>
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<tr>
<td>&quot; 20</td>
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<td>&quot;</td>
<td>1:30</td>
<td>1/2 hr.</td>
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<td>Nov. 15</td>
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<td>1</td>
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<td>1:50</td>
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<tr>
<td>Feb. 2</td>
<td>2</td>
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<td>1:50</td>
<td>20 mi.</td>
<td>20 mi.</td>
<td>Medium</td>
<td>Large</td>
<td>15 hr.</td>
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<td>&amp; Sedimentation</td>
<td>1:50</td>
<td>24 hr.</td>
<td>24 hr.</td>
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<tr>
<td>&quot; 20</td>
<td>4</td>
<td>Microscopical</td>
<td>1:50</td>
<td>1/2</td>
<td>1/2 hr.</td>
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<td>Slow</td>
<td>Small</td>
<td>22 hr.</td>
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</tr>
<tr>
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<td>1:50</td>
<td>24 hr.</td>
<td>24 hr.</td>
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</table>

The blood was examined just before I received the injection.

The tendency for the bacilli to clump was slight & the field was not cleared up.

Sedimentary reaction marked.

Wright's dead bacillary method was also employed with equally good result.

The serum gave no reaction when treated with the bacillus coli communis.
Following on the experiment performed on myself I next proceeded to vaccinate two guinea pigs; one against cholera and the other against typhoid.

The sera of neither of the animals, before receiving the injection, showed the reaction against the organisms.

The state of immunity was produced in each case, by following the lines laid down by Professor Pfeiffer (see Introduction).

Twenty one days after the guinea pigs had received their first dose of the virus a very marked sedimentary reaction was to be noticed. A photograph of the result will be seen on the accompanying page.

The immunity was so great in each case that complete degeneration of the organisms occurred, when brought in contact with their respective sera. The sera of these animals therefore showed a double action

1. An agglomerating - due to the presence of agglutinins.

2. A toxic action - causing the death and degeneration of the organisms, due to the presence of the alexines of Buchner.

It will thus be seen that substances can be produced within the body which have a specific action
on those organisms by means of which the immunity has been produced.

I cannot do better here than give a copy of the paper published by Max Gruber in the Lancet of October 9th 1897, on "The theory of active and passive immunity."

"1. A high degree of long persisting immunity can be obtained by means of intra-peritoneal injections (in guinea-pigs) of microbes killed either by chloroform or by exposure to a temperature 60°C. Such killed cultures of cholera, and other vibrios, of typhoid and coli bacilli, etc., have little or no poisonous properties; the guinea-pigs show trifling symptoms in the course of treatment; they recover rapidly, even when such large doses as 0.5 gramme per 1 kilogramme are eventually exhibited. The only constant symptoms arising from these injections are to be attributed to the peritonitis, which is caused by the proteins of the bacteria. It follows from these facts that the dead bodies of the bacteria are not poisonous in themselves; and, furthermore, that the immunising constituents of the bacteria are not identical with the bacterial toxins.

2. The animals, when immunised by this method
against the above-named bacteria, are truly infection-proof, but they are by no means toxin-proof. At the present time we are not dealing with toxin-proof immunity; and we are far from saying that animals cannot be rendered proof against the toxins of the above-named bacteria by the use of suitable methods.

3. The destruction of the bacteria takes place through the medium of the juices in actively immunised animals as well as in animals which are protected passively by means of the serums of immunised animals. This fact has been correctly observed and emphasised by Pfeiffer. The (polynuclear) phagocytes only play a secondary and comparatively unimportant part in the process.

4. Protective or antagonistic substances (antikörper) are always present in the blood and juices of the immunised animals. They are already formed and are not suddenly produced at the moment that a further inoculation is given, as has been asserted.

5. Both in actively and in passively immunised animals these substances (antikörper) react directly upon the bacteria, whether the contact occurs within the body or in vitro. There is no evidence that they undergo any changes or transformations in conjunction with the normal juices when animals are protected.
passively by their aid.

6. These protective substances are the characteristic constituent of the blood and juices of immunised animals. They are not capable of actually killing the bacteria by themselves.

7. The actual destruction of the bacteria is effected in all animals, whether actively or passively immunised, by means of the alexins of Buchner. These alexins are general protective substances entirely without specific action; they are universally present in all animals. Phagocytosis only takes a secondary share in the destruction.

8. The essential action of the protective substances of the blood and juices of immunised animals consists in making the bacterial cell walls adhesive. This is shown by the fact that the bacteria become sticky when treated by these juices; in consequence, they adhere together in clumps and lose their motility. We propose to call the specific antagonistic substances of immunised animals 'agglutinins.'

9. The agglutinins act upon the sheaths of the bacteria and make them more penetrable. The alexins are enabled thereby to reach the bacterial protoplasm and to destroy it — in other words, to kill the bacteria. This process takes place quite indifferently inside the living animal or in vitro,
the only condition necessary being that both agglutinin and alexins are present.

10. The agglutinins are used up during the process, perhaps by chemical combination or perhaps by actual destruction. It therefore follows that the extent of action of the juices of an immunised animal is directly proportional to the amount used.

11. Active immunity never occurs without evidence of the presence of agglutinin.

12. Active and passive immunity are identical in nature. Both forms of immunity depend upon the presence of agglutinins.

13. It has been asserted that active immunity persists even after the complete disappearance of the protective substances - that is to say, after the tissue juices have lost the power of conferring a specific passive immunity. This in reality is only the expression of the fact that the degree of concentration of the agglutinins gradually diminishes as time goes on; eventually the proportion of agglutinins present is insufficient to be effective in producing passive immunity.

14. I have been able to prove the presence of agglutinins thirteen months after the last immunising injection. How much longer they persist I am
unable to say, as at present I have not any animals which have been kept a longer time since their last treatment.

15. Agglutinins are specifically different. Each kind of bacterium has its own kind of agglutinin.

16. The influence of these specific agglutinins is, however, not limited specifically; it shows gradations in intensity of reaction, the maximum intensity of action being manifest upon its own kind. On other species the action is the more intense the more closely allied the microbe is to that by means of which the agglutinin was prepared.

17. The agglutinins are without doubt derived from certain constituents of the bacteria themselves (specific proteins?). They are produced only in the bodies of actively immunised animals, probably by combination with some constituents of the animal body. The site of production is perhaps in the macrophages; these cells ingest and destroy the polynuclear leukocytes which are laden with bacterial products.

18. The above conclusions are drawn from experiments with the microbes of cholera and allied vibrios, of typhoid fever, and the like. In diphtheria and tetanus other factors are probably present.
Other Diseases where the serum test may be employed as an aid to chemical diagnosis and the artificial production of agglutination of bacilli.

The cholera vibrios when brought in contact with the serum of a patient suffering from cholera show a similar reaction to that seen in enteric fever.

Pfeiffer was the first to point out the fact that the serum taken from a cholera convalescent and injected into a peritoneum of a non-immunised guinea pig along with an emulsion of cholera vibrios, exerted an influence on these organisms causing their degeneration etc. (See Introduction 'Pfeiffer's Phenomenon.') Gruber and Durham have corroborated this observation and performed the test 'in vitro' as well as 'in vivo.'

Professor MacFadyean of the Royal Veterinary College London has shown that the glanders bacillus reacts in the same way when brought in contact with the serum taken from a horse suffering from glanders.

Professor MacFadyean was kind enough to supply me with a sample of such a serum so that I was able to verify his observations. The glanders bacilli form into clumps exactly similar to the typhoid bacil-
This serum test will be of undoubted value in diagnosing those cases of glanders that occur in the human subject.

It is also of service in veterinary work. Although the mallein test is now universally used in testing for glanders in horses, it cannot be employed in those cases where death has occurred. It is on these occasions that the serum test will be of value as it can be performed with the serum taken from a dead animal.

Professor MacFadyean has found the test accurate, and an account of his work will be found in the Journal of Comparative Pathology and Therapeutics, December 1896.

The serum taken from pneumonic patients is said to exert an influence on the pneumococcus similar to that of an enteric serum on the Eberth bacillus.

Probably in a few years when our methods of working improve, this sero-diagnostic test may be of value in diagnosing other diseases such as diphtheria, syphilis, scarlet fever etc. No work has at present been done on this branch of the subject.

Regarding the artificial production of agglutination of bacilli, I have found an electric current is able to bring about this phenomenon.
Calcium carbonate according to Malvoz has a powerful agglutinating action on the Eberth bacillus; therefore care must be taken that this substance does not exist in the bouillon one uses for making the emulsion.

Lastly I may point out that normally, horses serum has a most marked agglomerating action on the typhoid bacillus.
I will here give a brief account of the results I have obtained in testing the sera of various patients.

Two hundred and forty-two cases have been examined and I have divided them into three groups, for the sake of convenience, as follows:

1. One hundred cases which were certainly enteric fever, owing to the clinical symptoms presented by the patients at the time of the examination of their sera, or which subsequently proved to be enteric.

2. One hundred and two cases which were certainly not enteric, owing to the clinical symptoms presented by the patients.

3. The examination of the sera taken from forty individuals who have at one time suffered from an attack of enteric fever; to ascertain how long after the disease the reaction can be obtained.

I will here take the opportunity of thanking the following gentlemen for the aid they have given
me in the obtaining of nearly all the above cases.

My best thanks are due to Dr Ker, Medical Superintendent of the City Hospital who has been kind enough to supply me with nearly all the cases of enteric fever reported on in this thesis. He has also allowed me to examine and report on the sera of many patients suffering from some of the other fevers, such as diphtheria, scarlet fever, measles etc.

I may also state that while I was acting as Resident at the City Hospital Dr Ker was often kind enough to corroborate the results I had obtained.

Through the kind permission of Sir Thomas Grainger Stewart, Dr Halliday Croom, and Dr Affleck, I have been able to examine and report on, cases suffering from diseases other than enteric. They form the bulk of the cases belonging to group 2.

For cases 31 to 53 inclusive I am indebted to Sir Thomas Grainger Stewart.

" " 59 & 64-69 inclusive I am indebted to Dr Halliday Croom.

" " 70-75 & 82-89 inclusive I am indebted to Dr Affleck.

My best thanks are also due to the Royal College of Physicians for allowing me the honour of working in
their Laboratory. It was here that I was able to carry on my experiments and perform the examinations of the sera of the various cases. Dr Noel Paton was often good enough to verify the results I obtained in the examination of my own serum after having been vaccinated.

Set I.

Cases where the clinical diagnosis of enteric fever was certain at the time of the examination of the blood serum, or where it was subsequently proved that enteric fever had existed.

Number of cases examined 100
Total number of observations 194

Methods employed for the Examination of the sera:

- Dr Muir's method 154 times
- McTaggart's dry blood method 22 "
- Professor Wright's sedimentation method or my own modification of his method 18 "

Total 194.
Dr Muir's method.

Positive results 140
Negative results 14
Total 154

McTaggart's method.

Positive results 17
Negative results 5
Total 22

Wright's Sedimentation method.

Positive results 18
Negative results 0
Total 18

Out of the 194 observations made

Total No. of positive results 175
" " " negative " 19
194

Of these 100 cases diagnosed as enteric fever
or proved afterwards to be enteric

97 gave a positive result during some
part of the course of the disease.
3 gave a negative reaction.

The three that gave no reaction were as fol-
Case No. 9 - which only happened to be examined once, by some oversight, and therefore might have given the reaction later on in the course of the disease; or on the other hand might have been one of those cases where on some days a positive result is obtained, while on others a negative.

Case No. 39. - which was examined three different times, on the 17th, 21st and 26th days of the disease. On none of these occasions was a positive result obtained, yet owing to the clinical symptoms presented one was justified in making a diagnosis of enteric fever.

Case No. 100. This is the most interesting one of the whole series and I have to thank Dr Gibson for his kindness in allowing me to report on it. He has not himself published the case yet but intends doing so. The diagnosis here was tubercular meningitis, and this was somewhat strengthened by the fact that no reaction could be obtained with the serum which was tested on four different occasions. The child ultimately died and on post mortem examination typical typhoid lesions were found in the intestines.

More curiously still, after death the Widal reaction was obtained. I think the reason that no
reaction was obtained during life may be explained in the following way. The dose of poison, that the child had had introduced into its system, was so great, and the vitality of the tissues so much diminished by this injection, that the body was incapable of reacting so as to be able to produce alexins or agglutinins. It is a harder matter however to account for the fact that the positive reaction was obtained in the blood after death.

Of the twenty two times that McTaggart's dry blood method was employed it will be noticed that on five of these occasions a negative result was obtained.

This is easily accounted for by the fact that the serum of these five cases had been preserved for three months and had therefore lost its agglutinative power.

In those cases examined by Dr Muir's method fourteen negative results were obtained.

Eight of fourteen negative examinations belong to the three negative cases just described.

In another case (No. 27) the examination was negative with a time limit of ½ hour, but was positive when a time limit of 1½ hours was employed.

In cases 33, 86 and 90 where negative results were obtained, the reaction became positive later on
in the course of the disease.

Lastly in case No. 15 - where a negative result was obtained on the 21st and 28th days of disease, a positive result was found on the 14th and 17th days of disease. This case left the hospital shortly after the last examination of the serum was made and was lost sight of, so it was impossible to ascertain if the reaction returned at some future date.

Temperature charts of the following cases, belonging to this group will be found at the end of this thesis:–

Nos. 1, 2, 3, 5, 7, 8, 10, 11, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 35, 53, 54 and 55.

Having dealt with the results obtained by the various methods employed one is able to compare the one with the other; and I am of opinion that of the three used, they are all equally reliable, but that Dr Muir's method is the most accurate. The only objection I have in using serum obtained from dry blood is that one is apt to have a large amount of debris in the specimen under examination, consisting of broken down red cells etc.

Instead of using blood serum for performing
the test various other fluids may be employed, such as:

1. Serum obtained from blisters.
2. Saliva.
3. Milk.
4. Urine.
5. Tears.

All these media have been found to give the reaction in patients suffering from enteric fever, however a longer time limit has to be employed as the reaction takes longer in appearing.

Of the 100 cases of enteric fever examined, the reaction was found to be present on the following days of disease:
Table of the days of the disease on which the reaction was obtained.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Days of Disease</th>
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<tbody>
<tr>
<td>2</td>
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The following is a table of the ages of the patients who gave the reaction.

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Set II.

The results of the Examination of the Sera of patients suffering from diseases other than Enteric Fever.

Number of cases examined 102
Total Number of Observations 194.

A previous attack of enteric fever was carefully enquired for in each case.

Methods employed for the Examination of the sera.

Dr Muir's Method 131 times.
McTaggart's dry blood method 47 "
Sedimentation method 16 "
Total 194.

Dr Muir's Method
Positive results 19
Negative results 112
Total 131
McTaggart's Method
Positive results  5
Negative results  42
Total  47

Sedimentation method
Positive results  0
Negative results  16
Total  16

Out of the 194 observations made
Total positive results 24
" negative " 170
194

Of these 102 cases which were certainly not suffering from enteric fever
88 gave complete negative results.
14 gave positive results.

Diseases in which the positive results were obtained are as follows.

1. Delirium tremens & acute rheumatism.
2. Pleurisy.
4. Ovarian tumor.
5. Pneumonia.
7. Acute rheumatism.
8. Psoas Abscess.
9. Puerperium (3 cases).
   and 3 cases in which the diagnosis was uncertain.
The most striking case where a positive result was obtained, was No. 5. She was a patient admitted into the Royal Maternity Hospital while I was resident there.

I will here give a short account of her case.

Mrs B. Age 27
Married. Housewife.
Admitted for her confinement to the R. M. H. Aug. 16/97, which occurred on Aug. 18th at 2.20 a.m.
She was very anaemic and had been so for 3 years.
Fourteen months previously she had been delivered of twins.
At the present delivery, the only special fact about the case was that the placenta was adherent and removed artificially. There was a good deal of post partum haemorrhage.
Douching was continued throughout the puerperium, as the lochia became foul, probably owing to two lacerations on the vulva. Her temperature chart is given at the end of this thesis. (Set II. No. 5. C.B)
Her serum was tested on the 1st, 2nd, and 4th of September and on each date a marked reaction was present. I was so astonished at the result and think-
ing that I must have introduced some fallacy, I sent
a sample of the serum to the College of Physicians
laboratory and the report that was sent to me con-
firmed my observation that a most marked reaction was
present.

I now enquired carefully into the history of
the patient for a recent or previous attack of en-
teric fever but could get nothing.

On examination of the patient — the abdomen
was flat, and soft, and she had no pain on palpation.
There were no rose spots to be seen. The tongue was
clean. The spleen was not enlarged. Diarrhoea was
present but that was owing to medicine she had receiv-
ed. The pupils were semi-dilated.

The patient left the hospital on the 8th Sep-
tember and I never obtained another chance of testing
her serum as she suddenly disappeared owing to family
matters.

Of course she may have been suffering from
an attack of ambulatory typhoid which would account
for the reaction being present.

Facts regarding the examination of the serum
will be seen in the tables at the end of the paper
Set II. No. 5.
The next cases worth noting are Nos. 1 & 4. They are also two puerperal cases. On examining their sera marked reactions were obtained. On both occasions other cases were examined with the same culture which gave negative results, showing that the culture was pure and in an active condition.

I am at a loss to know how to account for the reactions obtained in these three cases.

At first I thought that pregnancy or lactation might produce the necessary agglutinins, but this cannot be the reason as I have never again obtained the reaction in puerperal cases although I have tested several which are not recorded in this paper.

I have lately been led to believe that these positive results must be in a great measure due to the culture employed because it will be noted on looking at the tables that the positive results occur more or less in groups.

Fifteen of the twenty four positive results were obtained during September 1897 and the cultures used were reinoculated from each other. The remaining nine positive results were all obtained during February 1898 and one of the cultures employed I afterwards proved to be contaminated with some other or-
ganism.

I hold that it is of the greatest importance to be absolutely certain that one uses a pure active virulent culture.

The sera of cases 76 to 81 inclusive were also tested with the bacillus coli communis, and dead typhoid bacilli. No reaction was obtained in any of the cases.

Temperature charts of the following cases belonging to this set will be found at the end of this thesis. Nos. 1, 3, 4, 5, 11, 15.

Set III.

The results of the Examination of the Sera of subjects who have at one time of their life suffered from an attack of enteric fever.

My object in having gathered together this set of cases, was to ascertain how long after the disease the reaction could be obtained.

I had great difficulty in collecting these cases owing to the nomadic tendency of the ordinary
hospital patient. "For this reason many cases under observation at one time have been lost sight of.

I was very careful to obtain a definite history of enteric in each case.

Many of the cases, put down as having had enteric one year or six months previously, were cases which I had seen at the City Hospital while resident there and which had given a positive reaction during the attack.

In all 40 cases were examined and the following is a table showing how long after the disease a positive result was obtained.

<table>
<thead>
<tr>
<th>Case</th>
<th>Months after attack</th>
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<tbody>
<tr>
<td>26</td>
<td>1 month</td>
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<td>32</td>
<td>2</td>
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<td>26</td>
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</table>

From the above table it will be seen that I obtained a positive reaction in one case seven years after the attack. I took the precaution of having this blood examined also by another observer so as to be more certain of a true result being obtained.
M. Widal also publishes such a case that came under his notice.

The tables at the end, for this group of cases, give the various data regarding each case.

There is one case, No. 24, worthy of notice, which gave no reaction thirteen months after the attack, although before the reaction had been very marked.
Report of the Committee of the American Medical Association on the serum diagnosis of Typhoid Fever as given in the British Medical Journal, August 21, 1897.

1. In selecting the material used in making the test the choice between (a) serum, (b) dried blood, (c) fluid blood, and (d) blister fluid will depend largely upon whether the object be scientific research, clinical diagnosis in hospital or private practice, or public laboratory diagnosis where the samples have to be sent some distance.

2. In spite of considerable variation in technique, there has been a remarkable uniformity in the results obtained by those taking part in the discussion, and their average of about 95 per cent of successes agrees with the general average of the cases, nearly 4,000, thus far recorded in medical literature.

3. Each of the several methods of technique advocated may thus give good results in the hands of those thoroughly familiar with the details found necessary in each case and the sources of error to be avoided, success depending rather on being perfectly familiar with one method than on the particular one selected.

4. For routine diagnostic work even the very simplest
methods may give good practical results, but for recording scientific observations these methods which are accurately quantitative should be selected. This is especially necessary in reporting exceptional cases at variance with the general results recorded, or where the observations are made the basis of generalisations.

5. A complete reaction should comprise both characteristic clumping and total arrest of motion occurring within a definite time limit. For practical diagnostic work a dilution of 1 to 10, with a fifteen minute time limit, is convenient. In any doubtful case the dilution should be carried as far as 1 to 50, or perhaps 1 to 60, and a reaction not obtainable at that point should not be regarded as perfectly conclusive. For these higher dilutions the time limit should be extended to two hours.

6. Intensity of reaction in a given serum should be estimated by determining the degree to which it may be diluted without losing its power of giving a decided reaction, both as to agglutination and loss of motion.

7. The intensity of reaction shown by the same serum is influenced by the age, condition and virulence of the test culture and by the composition and reaction
of the culture medium. For purposes of comparison the sensitiveness of the test culture should be taken into consideration.

8. The evidence so far recorded establishes that the reaction may be delayed or occasionally may not be obtained in cases of genuine typhoid infection; and also that it may be exceptionally present in non-typhoid cases, though not in an intense degree.

9. In investigating exceptional and contradictory results the following circumstances have to be considered: (a) The uncertainty of clinical diagnosis; (b) the absence of bacteriological or other confirmatory methods of diagnosis during life, giving decisive negative results; (c) the possibility of overlooking typhoid infection, even post mortem, in the absence of characteristic intestinal lesions where a very thorough bacteriological examination has not been carried out.

10. The modifying influences mentioned above suffice to explain the divergencies existing in the reports of different observers. Without being absolutely infallible the typhoid reaction appears to afford as accurate diagnostic results as can be obtained by any of the bacteriological methods at our disposal for the diagnosis of other diseases. It must cer-
tainly be regarded as the most constant and reliable sign of typhoid fever, if not an absolute test.
CONCLUSIONS.

I. That the serum test is of undoubted value as an aid to clinical diagnosis in enteric fever, and especially in those cases where a diagnosis is often uncertain.

I cannot agree with Professor Thomson of New York University who states that "as a genuine diagnostic aid, the test has about the value of the diazo reaction in typhoid urine, or the study of leucocytosis in pneumonia, that is, it is confirmatory in connection with appropriate symptoms but misleading if positive reliance be placed upon it."

Many cases that I have tested, where a positive reaction was obtained and the diagnosis uncertain have afterwards proved to be enteric fever (See Report on Cases).

II. The test undoubtedly fails in about two or three per cent of cases where it should be found present. Whether this percentage of negative results is due to errors introduced from without or is due to the non-production of agglutinins in the blood is at present unknown.

Again diseases other than enteric occasionally
give a positive result. The cause of this is also unknown.

Some observers have placed the percentage of negative results as high as twenty three. I have not found this to be the case and think that many wrong results are due to inaccuracy in methods of working.

III. Instead of employing blood serum for the test other media may be used such a fluid obtained from blisters, milk, urine, tears and saliva.

Blood serum however gives the most reliable results.

IV. In performing the test it is absolutely necessary that a pure virulent culture of the Eberth bacillus be employed as agglomeration of bacilli is apt to occur in the presence of a non typhoid serum if an attenuated culture be used.

The culture should be from 15 to 24 hours old and certainly never more than 48 hours old.
(See note on typhoid culture.)

V. The dilution of the serum should be about 1 in 30 as many observers have pointed out that often diseases other than typhoid give a positive re-
action when a concentrated serum is used.

A standard dilution for the medium depends on whether one employs blood serum, dried serum, blister fluid, milk, urine, etc.

The dilution must be less when media other than blood serum are used.

VI. The time limit necessary for an observation which is microscopical depends on the medium used and its dilution.

If using fluid blood serum and a dilution of 1 in 30 I have found a time limit of \( \frac{3}{4} \) hour ample for giving accurate results.

If the dilution is less then a shorter time limit must be used and vice versa.

It is unnecessary to carry on an observation for two or three hours as some advocate, when employing a dilution of 1 in 30 and a pure virulent culture (See note on dilution of serum and time limit.).

VII. The reaction as presented by the action of a typhoid serum on the Eberth bacillus consists of

1. Loss of motility of bacilli.
2. Clump formation - or agglomeration of bacilli.
3. Degeneration of bacilli.

It is not necessary for every enteric serum to show these 3 actions. The first two may exist independently of the third.
VIII. A complete reaction is hardly ever absent after the seventh day of disease but is often present at a much earlier date.

A positive reaction may always be obtained during the first week of the disease, and the presence of it is a strong proof of the existence of enteric fever.

An incomplete reaction may be due to the following causes.

1. Too great a dilution of the serum
2. The employment of an attenuated culture.
3. The production of only a small amount of agglutinins in the blood.
4. The destruction of the agglutinins by exposure of the serum to various surrounding influences, such as heat, cold etc.

The reaction occurs best at a normal room temperature. Surrounding influences have to be taken into consideration regarding the rapidity with which the reaction occurs.

It is always advisable to collect the blood which has to be examined in new tubes which have never been used so that the risk of contaminating a serum may be avoided. All glass cells and instruments which have to be used for other examinations should be kept thoroughly clean and aseptic.

The method I employ for cleaning and ren-
dering aseptic any instrument is to first wash thoroughly in water then pass through solutions of lysol, methylated spirit, and methylated ether.

IX. The reaction may be present on one day of the disease and absent on the next therefore a blood examination should be made on several consecutive days.

X. Control experiments should be always carried out in those cases where a doubtful reaction occurs, and it is advisable always to examine ones culture before using it, by making an emulsion of the bacilli in bouillon and examining a drop under the microscope.

XI. Of the various methods for performing the test Dr Muir's modification of Grünbaum's method gives the most accurate results, and is also easily performed.

The instruments used for this method are certainly more expensive than those employed for others but then greater accuracy is obtained and I hold that this is an essential for the test.
Certainly in performing the dry blood method, serum can be more easily transmitted by post from a distance, but then I think there is a danger of the serum becoming contaminated or of losing its agglutinative power.

Artificial heat must not be used to dry the blood or the agglutinins will be destroyed, and in my experience I have found that a drop of blood on a piece of paper which is non-absorbent takes a considerable time to dry.

If blood has to be transmitted by post or otherwise, I think the best method is to send it sealed in sterile blood capsules similar to those used by Professor Delépine.

I have found that receiving blood in such capsules one is able to make more accurate dilutions of the serum than with dry blood.

It would be quite simple for a central laboratory to keep a stock of such capsules ready for use and send them out to any practitioner who might need them.

Regarding the sedimentary methods I find Professor Wright's tubes hard to manipulate; and owing to the calibre of the stem of the pipette being so small, the column of the fluid
is not broad enough to be able to note slight degrees of turbidity.

The modified sedimentary tubes I have adopted are more easily manipulated and the column of fluid being 1/16 inch in thickness more accurate results are obtained. Again I have a control tube close beside the other and results can very easily be compared.

In using dead bacilli for the sedimentation test great care must be taken not to mistake the fine granular precipitate consisting of these bacilli with the floccular precipitate of a true reaction. For this reason I think it advisable always to use live bacilli.

The precipitate of dead bacilli is also apt to conceal from view small clumps which are formed in a partial reaction.

The sedimentation test takes twenty four hours to perform while the whole observation can be finished in one hour by employing one of the microscopical methods.

XII. The serum test is available as an aid for diagnosis in certain other diseases; using the specific microbe of that disease for the test in the
place of the Eberth bacillus.

At present it is of use in diagnosing cases of cholera, pneumonia, and glanders.

As our knowledge increases and our methods improve the test will in all probability be of use as a diagnostic aid for other diseases besides those mentioned such as diphtheria, gonorrhea, scarlet fever, syphilis etc. The test may ultimately be used in order to discover the specific organisms of certain diseases such as scarlet fever, syphilis etc, by watching the action of a serum taken from patients suffering from these diseases on organisms cultivated from the discharges etc. of such patients.

XIII. The phenomenon of agglomeration of typhoid bacilli may be produced artificially by the action of certain drugs, electrical currents, or the action of serum taken from the horse, upon them.

Horses serum has a powerful and rapid agglomerating action on the Eberth bacillus.

XIV. It is possible to produce a high state of immunity in the human subject by the method of vaccination as proposed by Mr Haffkine and Professor
Wright with such organisms as the cholera vibrio or the Eberth bacillus.

This method of vaccination may act as a check on the ravages produced by such diseases in those countries where they are most prevalent.

XV. The power of agglutination may be retained by a serum for a number of years after a patient has had an attack of enteric fever.

I have obtained one serum which gave a marked reaction seven years after the disease.

M. Widal has also published such a case where the reaction was positive after this lapse of time.

XVI. During an attack of enteric fever, or in the production of artificial immunity in a subject, certain substances are produced in the blood, which have an antagonistic action on the Eberth bacillus.

1. Agglutinins - which are the essentials for causing agglomeration of bacilli as demonstrated in a reaction.

2. Alexins of Buchner - which are the toxic products causing degeneration and destruction of the bacilli.

It is not necessary for both products to be
present in the blood at the same time. Sometimes the alexins are absent while the agglutinins are present, on the other hand the agglutinins are never absent when the alexins are present. For this reason I hold that the agglutinins are the first products formed in the blood after infection has occurred, the alexins being produced later.

XVII. The age or sex of a patient has no effect on the production of alexins or agglutinins, nor does the severity of the case or the temperature of the patient appear to have an effect on the intensity of the reaction.

XVIII. From the experiments performed by myself I am able to assert that an enteric serum has no action on the bacillus coli communis, and that the serum test is of undoubted value as an aid for differentiation between the Eberth and Escherich groups of bacilli.

Durham has shown that the Gartner group of bacilli, which are more closely related to the Eberth group do give a positive reaction in the presence of a typhoid serum.
I am unable to speak on this point as I have not been able to experiment with the Gartner bacillus, not having had a culture at my disposal.
One hundred cases which were certainly enteric fever, owing to the clinical symptoms presented by the patients at the time of the examination of their sera, or which subsequently proved to be enteric.
<table>
<thead>
<tr>
<th>No. of Case</th>
<th>Name</th>
<th>Age</th>
<th>Date when serum was examined.</th>
<th>Morning &amp; Evening Temperature</th>
<th>Method of examination of serum</th>
<th>Dilution of serum</th>
<th>Number of observations</th>
<th>Time limit of examination of serum</th>
<th>Reaction present or absent</th>
<th>Activity of bacilli</th>
<th>Rapidity of Agglomeration</th>
<th>Size of clumps.</th>
<th>Diarrhoea</th>
<th>Abdomen</th>
<th>Splen.</th>
<th>Rose spots.</th>
<th>Previous attacks</th>
<th>Age of culture</th>
<th>Remarks</th>
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<td>M</td>
<td>Dec 1897. Mebr. 17.</td>
<td>M. 98</td>
<td>Fluid</td>
<td>1:20</td>
<td>1</td>
<td>15 m. x</td>
<td>Absent</td>
<td>Rapid</td>
<td>V.Large</td>
<td>Dist.</td>
<td>Enl.</td>
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<td>2</td>
<td>J.G 24</td>
<td>M</td>
<td>17. 12</td>
<td>M. 100.4</td>
<td>Fluid</td>
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<td>1</td>
<td>30 m. x</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Medium</td>
<td>&quot;</td>
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<td>Apr. 4</td>
<td>M. 97.8</td>
<td>Fluid &amp; dry</td>
<td>1:20</td>
<td>1</td>
<td>30 m. x</td>
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<td>F</td>
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<td>M. 102</td>
<td>Fluid</td>
<td>1:20</td>
<td>1</td>
<td>30 m. x</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Slow</td>
<td>x</td>
<td>Enl.</td>
<td>&quot;</td>
<td>&quot;</td>
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<td>M. 100.6</td>
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<td>1</td>
<td>30 m. x</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Rapid</td>
<td>Large</td>
<td>&quot;</td>
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<td>W.H. 8</td>
<td>M</td>
<td>17. 32</td>
<td>M. 98.6</td>
<td>Fluid</td>
<td>1:20</td>
<td>1</td>
<td>30 m. x</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Medium</td>
<td>&quot;</td>
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<tr>
<td>7</td>
<td>G.J 44</td>
<td>M</td>
<td>17. 41</td>
<td>M. 98.4</td>
<td>Fluid</td>
<td>1:20</td>
<td>1</td>
<td>30 m. x</td>
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<td>E.L. 9</td>
<td>M</td>
<td>17. 7</td>
<td>M. 101.1</td>
<td>Fluid</td>
<td>1:20</td>
<td>1</td>
<td>30 m. x</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Large</td>
<td>x</td>
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<td>9</td>
<td>M.M 11</td>
<td>F</td>
<td>17. 18</td>
<td>M. 97.6</td>
<td>Fluid</td>
<td>1:20</td>
<td>1</td>
<td>30 m. x</td>
<td>Present</td>
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<tr>
<td>10</td>
<td>M.H. 7</td>
<td>F</td>
<td>17. 14</td>
<td>M. 101.6</td>
<td>Fluid</td>
<td>1:20</td>
<td>1</td>
<td>30 m. x</td>
<td>Absent</td>
<td>Rapid</td>
<td>Large</td>
<td>&quot;</td>
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<tr>
<td>11</td>
<td>M.E. 13</td>
<td>F</td>
<td>17. 14</td>
<td>M. 102.4</td>
<td>Fluid</td>
<td>1:20</td>
<td>1</td>
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Remarks:
- Spots seen on 10, 11, 14, 16, day of illness.
- Death of patient.
- Samum oris.
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- **Remarks:** Blood tested during 3rd week of convalescence.
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**Method of examination of Serum**

**Dilution of Serum**

- 1:50
- 1:20
- 1:10

**Number of observations.**

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**Time limit of examination of serum**

- 20 min.
- 40 min.
- 60 min.

**Reaction present x absent o**

**Motility of bacilli.**

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**Rapidity of Agglomeration.**

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**Size of clumps.**

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<th>Flat</th>
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<td>M. 102.3</td>
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**Diarrhoea.**

<table>
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<tr>
<th>Abdomen.</th>
<th>Spleen.</th>
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**Rose spots.**

<table>
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<tr>
<th>Previous attacks</th>
<th>Age of culture employed</th>
</tr>
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<tbody>
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<td>M. 102.3</td>
<td>M. 102.3</td>
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</table>

**Complications**

**Remarks.**
<p>| No. of Case | Name | Age | Sex | Date when serum was examined | Dilution of serum | Method of examination of serum | Time limit of reaction present x 100 | Motility of bacilli | Rapidity of agglomeration | Size of clumps | Diarrhoea | Abdomen | Splene | Rose spots | Previous attacks | Age of culture employed | Complications | Remarks |
|------------|------|-----|-----|------------------------------|-----------------|-----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|----------|--------|---------|-------------|------------------|-----------------|-----------------|-----------|--------|
| 54         | J.B  | 15  | M   | 1897 Sep. 21                  | Fluid           | M. 99.3 E.101.4 | 30 m x             | Present           | Slow            | Small           | Flat | Enl. | -       | 115hr     | -                | -               |
| 55         | E.G  | 20  | M   | 1897 Sep. 30                  | Fluid           | M. 99.8 E.102   | 60 m               | Present           | Small x         | Dist             | ?    | -    | -       | subsus-tus tendinum | -               | -               |
| 56         | D.C  | 10  | M   | 1897 Sep. 18                  | Fluid           | M. 99.6 E.101.2 | 15 m               | Absent Rapid      | Medium         | -                | -    | -    | -       | -            | -               | -               |
| 57         | E.C  | 16  | F   | 1897 Sep. 18                  | Fluid           | M. 99 E.100.6   | 15 m               | Present           | Large           | Not enl.        | -    | -    | -       | Purpura     | -               | -               |
| 58         | E.S  | 8   | F   | 1897 Sep. 30                  | Fluid           | M. 98 E.98.4    | 30 m               | Present           | Not enl.        | -                | 24hr P.M. | -    | -       | Bronchitis | Death No P.M. | -               | -               |
| 59         | A.B. | 4   | M   | 1897 Oct. 10                  | Fluid           | M.100.1 E.101.6 | 45 m               | Present           | Small           | -                | 24hr Abscess of scalp | 15hr  | -    | -       | Bronchitis   | -               | -               |
| 60         | M.K  | 25  | F   | 1897 Oct. 10                  | Fluid           | M.102.6 E.102.8 | 11 m               | Present           | Small           | -                | -    | -    | -       | Perforation  | -               | -               |
| 61         | P.T  | 26  | M   | 1897 Oct. 10                  | Fluid           | M.103.8 E.104    | 15 m               | Absent Rapid     | Medium           | -                | 24hr P.M revealed typh. lesions | -    | -    | -       | -            | -               | -               |
| 62         | M.J  | 7   | F   | 1897 Oct. 10                  | Fluid           | M.102 E.104.4   | 30 m               | Present           | Small x         | Not enl.        | Flat | Enl. | 15hr    | Bronchitis | -               | -               |
| 63         | N.M  | 6   | F   | 1897 Oct. 10                  | Fluid           | M.100 E.101     | 20 m               | Present           | Medium          | -                | 115hr| -    | -       | -            | -               | -               |
| 64         | G.R  | 5   | F   | 1897 Oct. 10                  | Fluid           | M.102 E.103.8   | 15 m               | Absent Rapid     | Large           | -                | -    | -    | -       | -            | -               | -               |
| 65         | T.S  | 11  | M   | 1897 Oct. 10                  | Fluid           | M. 98 E.98.4    | 30 m               | Present           | Medium          | Flat | Enl. | 15hr    | Bronchitis | -               | -               |
| 66         | W.V  | 11  | M   | 1897 Oct. 10                  | Fluid           | M.100.4 E.102.5 | 60 m               | Present           | Medium          | -                | 15hr| -    | -       | -            | -               | -               |</p>
<table>
<thead>
<tr>
<th>No. of Case</th>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>Date when serum was examined</th>
<th>Morning &amp; Evening Temperature</th>
<th>Method of examination of Serum</th>
<th>Dilution of Serum</th>
<th>Number of observations</th>
<th>Time limit of serum examination present</th>
<th>Reaction present</th>
<th>Motility of bacilli</th>
<th>Rapidity of agglomeration</th>
<th>Size of clumps</th>
<th>Diarrhea</th>
<th>Abdomen</th>
<th>Spleen</th>
<th>Rose spots</th>
<th>Previous attacks</th>
<th>Age of culture employed</th>
<th>Complications</th>
<th>Remarks</th>
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<td>14</td>
<td>M</td>
<td>1871 Oct 21 16</td>
<td>M.102.4 E.103.2 M.98.4 E.98.6</td>
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<td>1-30</td>
<td>3</td>
<td>30 m x</td>
<td>Absent</td>
<td>Rapid</td>
<td>Large x</td>
<td>Dist. Enl.</td>
<td>-</td>
<td>-</td>
<td>15 hr</td>
<td>-</td>
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<td>3</td>
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<td>Absent</td>
<td>Rapid</td>
<td>Large ?</td>
<td>? x</td>
<td>15 hr</td>
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<td>Phlebitis</td>
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<td>3</td>
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<td>Absent</td>
<td>Slow</td>
<td>Medium ?</td>
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<td>22 10</td>
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<td>3</td>
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<td>Rapid</td>
<td>Large ?</td>
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<td>4</td>
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<td>&quot;</td>
<td>Slow</td>
<td>Medium x</td>
<td>? x</td>
<td>15 hr</td>
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<td>3</td>
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<td>&quot;</td>
<td>&quot;</td>
<td>Medium x</td>
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<td>&quot;</td>
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<td>?</td>
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<td>Day of Disease</td>
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<td>Agglutination</td>
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<td>Medium</td>
<td>Large</td>
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<td>x</td>
<td>Not</td>
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<td>x</td>
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<td>Age</td>
<td>Sex</td>
<td>Date when Serum was examined</td>
<td>Date of Disease</td>
<td>Morning &amp; Evening Temperature</td>
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<td>Dilution of Serum</td>
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<td>Rapidity of Agglomeration</td>
<td>Size of Clumps</td>
<td>Diarrhoea.</td>
<td>Adenon.</td>
<td>Spleen</td>
<td>Rose Spots.</td>
<td>Previous Attacks</td>
<td>Age of culture employed</td>
<td>Complications</td>
<td>Remarks</td>
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<td>18</td>
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<td>1-30</td>
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<td>Absent</td>
<td>Rapid</td>
<td>Large</td>
<td>Not</td>
<td>?</td>
<td>x</td>
<td>24hr</td>
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<td>6</td>
<td>Dec. 19</td>
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<td>60 m</td>
<td>4</td>
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<td>Slow</td>
<td>Small</td>
<td>Not</td>
<td>Not</td>
<td>-</td>
<td>-</td>
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<td>Reaction was very incomplete.</td>
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<td>Jan. 22</td>
<td>24</td>
<td>?</td>
<td>1897 E. 98</td>
<td>M. 97</td>
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<td>5</td>
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<td>Slow</td>
<td>Small</td>
<td>Not</td>
<td>Enl.</td>
<td>-</td>
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<td>4</td>
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<td>30 m</td>
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<td>Present</td>
<td>-</td>
<td>Dist.</td>
<td>Enl.</td>
<td>x</td>
<td>-</td>
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<td>30 m Absent</td>
<td>4</td>
<td>Absent</td>
<td>Slow</td>
<td>Medium</td>
<td>-</td>
<td>x</td>
<td>-</td>
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<td>Clumps arranged in form of reticulum.</td>
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<td>8</td>
<td>M. 101.8</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>Dead bacilli were also used with same result.</td>
<td>-</td>
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<td>Age</td>
<td>Sex</td>
<td>Date when serum was examined</td>
<td>Day of disease</td>
<td>Morning &amp; Evening Temperature</td>
<td>Method of Examination of Serum</td>
<td>No. of observations</td>
<td>Time limit of Examination of serum</td>
<td>Reaction</td>
<td>Presence</td>
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<td>Motility of bacilli</td>
<td>Rapidity of Agglomeration</td>
<td>Size of clumps</td>
<td>Diarrhoea</td>
<td>Abdomen</td>
<td>Splen.</td>
<td>Rose Shots</td>
<td>Previous Attacks</td>
<td>Age of culture employed</td>
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<td>M</td>
<td>Feb. 11</td>
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<td>M.103.4 E.102.2</td>
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<td>2 hrs.</td>
<td>Present</td>
<td>-</td>
<td>- 2 hrs.</td>
<td>- 2 hours</td>
<td>- 2 hours</td>
<td>- 2 hours</td>
<td>- 2 hrs.</td>
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<td>M</td>
<td>25</td>
<td>M</td>
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<td>M.101.6 E.102.6</td>
<td>Fluid</td>
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<td>24 hrs.</td>
<td>Absent</td>
<td>-</td>
<td>- 24 hrs.</td>
<td>- 24 hrs.</td>
<td>- 24 hrs</td>
<td>- 24 hrs</td>
<td>- 24 hrs</td>
<td>Flat Enl.</td>
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<td>21</td>
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<td>-</td>
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<td>- 15 min.</td>
<td>- 15 min.</td>
<td>- 15 min</td>
<td>- 15 min</td>
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<td>Slow</td>
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<td>-</td>
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<td>- 2 hrs</td>
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<td>-</td>
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<td>The blood gave the reaction after death. P.M. revealed typhoid lesions in intestine.</td>
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SET II.

One hundred and two cases which were certainly not enteric, owing to the clinical symptoms presented by the patients.
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<th>Name</th>
<th>Diagnosis</th>
<th>Date when Serum was examined</th>
<th>Age</th>
<th>Sex</th>
<th>Method of examination of Serum</th>
<th>Dilution of Serum</th>
<th>Time limit of examination of Serum</th>
<th>Reaction present</th>
<th>Motility of Bacilli</th>
<th>Rapidity of Agglomeration</th>
<th>Size of Clumps</th>
<th>Age of culture employed</th>
<th>Complications and Remarks</th>
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<td>Puerperium</td>
<td>1897 Sep.17</td>
<td>33</td>
<td>F</td>
<td>Fluid</td>
<td>1-30</td>
<td>30 m x</td>
<td>Absent</td>
<td>Rapid</td>
<td>Medium</td>
<td>28hr</td>
<td>Lochial discharge was also employed as well as the other methods, &amp; a positive result was obtained.</td>
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<td>A.F.</td>
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<td>17 M. 96</td>
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<td>Fluid</td>
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<td>3hr. - Present</td>
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<td>2</td>
<td></td>
<td></td>
<td>Present</td>
<td>Slow</td>
<td>Medium</td>
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Antitoxin was given.
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<th>No. of Observations</th>
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<th>Age of Disease</th>
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This serum was also tested in a dilution of 1 in 10, and no clumping or loss of motility of bacilli occurred. Time limit 2 hours.
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The serum was also tested in a dilution of 1 in 10, and no clumping or loss of motility of bacilli occurred. Time limit 2 hours.
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</table>

P.M. revealed no typhoid lesions.

The culture employed in cases 60, 61, 62, 63, & 69 was infected with some other organism. When grown on gelatine liquefaction of the medium occurred.

The culture employed in cases 60, 61, 62, 63, & 69 was I believe infected with some other organism. When grown on gelatine liquefaction of the medium occurred.
<table>
<thead>
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<th>No. of Case</th>
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<th>Rapidity of Agglomeration</th>
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<td>44</td>
<td>F</td>
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SET III.

The examination of the sera taken from forty individuals who have at one time suffered from an attack of enteric fever; to ascertain how long after the disease the reaction can be obtained.
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Extreme activity of movement of bacilli at end of observation.

This case was also tested by another observer to control my result.

Cases 13 to 17, and 19 to 22 gave the same reaction with the dry as with the fluid serum.
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<th>Sex</th>
<th>Date of Examination</th>
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<th>Method of examination</th>
<th>Dilution of Serum</th>
<th>No.of observations</th>
<th>Time limit</th>
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<td>M</td>
<td>&quot;</td>
<td>2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1</td>
<td>&quot; Slow</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>29</td>
<td>T.S.</td>
<td>26</td>
<td>F</td>
<td>Mch. 1</td>
<td>13 mths.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1</td>
<td>&quot; Present</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>30</td>
<td>J.S.</td>
<td>6</td>
<td>M</td>
<td>&quot;</td>
<td>1</td>
<td>13 mths.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1</td>
<td>&quot; Present</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>31</td>
<td>A.N.</td>
<td>21</td>
<td>F</td>
<td>&quot;</td>
<td>1</td>
<td>1 yr.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1</td>
<td>&quot; Absent</td>
<td>Medium Medium</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>32</td>
<td>A.G.</td>
<td>1</td>
<td>F</td>
<td>&quot;</td>
<td>7</td>
<td>1 yr.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1/2</td>
<td>&quot; Rapid</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>33</td>
<td>M.S.</td>
<td>3</td>
<td>F</td>
<td>&quot;</td>
<td>7</td>
<td>1</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1</td>
<td>&quot; Present</td>
<td>Slow</td>
<td>Small</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>34</td>
<td>G.M.</td>
<td>13</td>
<td>F</td>
<td>&quot;</td>
<td>7</td>
<td>2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>35</td>
<td>D.H.</td>
<td>24</td>
<td>M</td>
<td>&quot;</td>
<td>11</td>
<td>5 mths.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>15 min.</td>
<td>&quot; Absent</td>
<td>Rapid</td>
<td>Large</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>36</td>
<td>S.S.</td>
<td>16</td>
<td>M</td>
<td>&quot;</td>
<td>14</td>
<td>4 yrs.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1 1/2 hr.</td>
<td>&quot; Present</td>
<td>Slow</td>
<td>Small</td>
<td>20</td>
<td>&quot;</td>
</tr>
<tr>
<td>37</td>
<td>A.E.</td>
<td>35</td>
<td>F</td>
<td>&quot;</td>
<td>14</td>
<td>29 yrs.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1/2</td>
<td>&quot; -</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>38</td>
<td>M.E.</td>
<td>46</td>
<td>F</td>
<td>&quot;</td>
<td>14</td>
<td>29</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1/2</td>
<td>&quot; -</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>39</td>
<td>M.S.</td>
<td>28</td>
<td>F</td>
<td>&quot;</td>
<td>18</td>
<td>3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1</td>
<td>x Absent</td>
<td>Medium Small</td>
<td>19</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>40</td>
<td>H.P.</td>
<td>32</td>
<td>M</td>
<td>&quot;</td>
<td>18</td>
<td>6 mths.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1/2</td>
<td>&quot; Rapid</td>
<td>&quot;</td>
<td>&quot;</td>
<td>19</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

This was a case of ambulatory typhoid which showed the test markedly 13 months previously.

The 2nd observation was made with dead bacilli, with an equally good result.
1. Serum of Scarlet fever patient.
   Q.D. Age 10. 22/2/98.
   No sedimentation.
   Dilution of Serum 1 in 40.

2. Serum of Enteric Fever patient.
   D.T. Age 20. 22/2/98
   Marked sedimentation.
   Supernatant fluid clear.
   Dilution of Serum 1 in 40.
1. Serum of patient suffering from phlebitis.
   E.C. Age 44. F. 19/2/98.
   Dilution of Serum 1 in 30. No sedimentation. Uniform turbidity of fluid.

2. Serum of Enteric Fever patient.
   R.M. Age 25. M. 19/2/98
   Dilution of Serum 1 in 30. Marked sedimentation, supernatant fluid clear.
1. Serum of an Enteric Patient showing sedimentation. Dilution 1 in 30.
2. Control Tube. Serum of Scarlet Fever patient, showing turbidity throughout whole column of fluid. Dilution 1 in 30.

la. Serum taken from horse. Dilution 1 in 50.
lb. Serum of Enteric patient. Dilution 1 in 50.

Both tubes show marked sedimentation.

All four tubes incubated for 24 hours.
1. Serum taken from a typhoid immunised guinea pig.
   Showing marked sedimentary reaction. Dilution 1 in 400.

2. Serum taken from a cholera immunised guinea pig.
   Showing same reaction when cholera vibrios are mixed with it.
   Dilution 1 in 400.
Serum taken from a normal Guinea Pig.

Dilution of serum in each tube 1 in 100.

1. Inoculated with the bacillus Typhosus.
2. Inoculated with the Cholera Vibrio.

After standing for 48 hours, no sedimentation has occurred, and the supernatant fluid is distinctly turbid in each case.
1. Normal Human Serum.
2. Serum of Enteric Patient.

Dilution of the Sera 1 in 50.

Both inoculated with the Bacillus Coli Communis.

Showing marked turbidity and no sedimentation after being incubated for 24 hours.
Temperature Charts.

Set I. No. 1. D.S.

Set I. No. 2. J.G.

Set I. No. 5. H.T.
Temperature Charts.

Set I. No. 3. M.R.

Set I. No. 7. G.J.

Set I. No. 8. L.L.
Temperature Charts.

Set I. No. 18. W.P.

Set I. No. 19. T.L.

Set I. No. 20. R.C.
Temperature Charts.

Set I. No. 28. A.S.

Set I. No. 35. J.C.

Set I. No. 53. R.D.
Temperature Charts.

Set I. No. 54. N.R.

Set I. No. 55. M.H.
Temperature Charts.

Set II. No. 1. J.W.

Set II. No. 3. J.

Set II. No. 4. C.J.
English and American Literature.

1. H. E. Durham

2. F. Widal.

3. A. Sheridan Delépine, M.D.

4. W. Johnston, M.D.

5. A. S. Grünbaum.


7. Sheridan Delépine.
   "On the Sero-Diagnosis of Typhoid Fever."
   " " " " Part II, " 12.1896. (p.1665.

8. Professor MacFadyean.

9. A. E. Wright, M.D.
   "Note on the Technique of Serum Diagnosis of acute Specific Fevers." British Medical Journal, Jan. 16th 1897, p.139.

10. McWeeney.
11. A. E. Wright and Surgeon Major Semple.  
"Remarks on Vaccination against Typhoid Fever"  

"On the value of the different bacteriological methods of diagnosis of typhoid fever."  
The Medical Chronicle. vol. vii. March 1897.

13. A. E. Wright and F. Smith.  
A note "on the occurrence of Malta Fever in India."  

14. A. Sheridan Delépine.  
"The technique of the 'serum-diagnosis' with special reference to typhoid fever."  

15. A. S. Grünbaum.  
"Remarks on Method in serum diagnosis."  

16. A. E. Wright and D. Semple.  
"On the employment of dead bacteria in the serum diagnosis of typhoid and Malta fever."  

17. E. T. Fison.  
"Widal's Sero-Diagnosis of typhoid fever."  

18. H. Batty Shaw.  
"Widal's reaction on the infant child of a mother who during gestation had contracted typhoid fever."  

19. Max Gruber.  
"A theory of active and passive immunity from the bacteria of cholera, typhoid fever and the like."  

"The examination of one hundred cases of typhoid fever by Widal's serum test."  

"Notes on the Examination of the blood (1) Serum diagnosis (2) A form of haemacytometer."  
The Edinburgh Medical Journal.  
22. F. Widal.
"The serum diagnosis of Typhoid Fever."

23. S. R. Christophers.
"Note on the specific Action of normal human serum upon the bacillus coli communis."

"On the serum Diagnosis of typhoid fever with especial reference to the Bacillus of Gärtner and its allies."

25. Claude B. Ker.
"The serum diagnosis of Enteric Fever."
The Scottish Medical and Surgical Journal.
French Literature.

1. Charrin & Roger.
   "Note on the development of pathogenic organisms in the serum of vaccinated animals."

2. E. Metchnikoff.

3. E. Metchnikoff.
   "The actual state of the question of immunity." Annales de l'Institut Pasteur. 1894. vol. viii. (pp. 706-721.)

4. E. Metchnikoff.

5. J. Bordet.

   "On the mode of action of preventive Sera" Annales de l'Institut Pasteur. 1896. vol. x. (pp. 193-219.)
   (see also vol. vi.)

   "Immunity against the virus of Typhoid Fever" Annales de l'Institut Pasteur. v. ii. p. 54. (1888.)

8. F. Widal.
   Semaine Médicale. July 1, 8, 29, 1896.
   Bulletin Médical. 618-619. 1896
   "  766-768. 1896
   La Presse Médicale. 253. 1896.


10. Achard.

11. Achard & Bensaude.
12. Chantemesse.  
    Semaine Médicale.  p. 303.  1896.


15. Courmont.  


17. Widal & Chantemesse.  
    Annales de l'Institut Pasteur. vol. vi. p. 754.
German Literature.

1. Behring & Nissen.
"Bactericidal Properties of various kinds of blood sera."
Zeitschrift für Hygiene. 1890. v. viii. (pp. 412 - 432.

2. R. Pfeiffer.
"Research on the nature of cholera immunity and the specific bactericidal action."
Zeitschrift für Hygiene. 1894. v. xviii. p. 1

3. R. Pfeiffer.
"The Differential Diagnosis of the Vibrios of Asiatic Cholera with the help of Immunising."
Zeitschrift für Hygiene. 1895. v. xix. pp75-100.

"On the Specific Immunising Reaction of Typhoid Bacilli."
Zeitschrift für Hygiene. 1896. v. xxii. (pp. 203 - 246.

5. Max Gruber.
Münchener Medicinische Wochenschrift. 1895. vi.

6. Max Gruber.

7. Stern.
Deutsche Medicinische Wochenschrift. 1892. no. 37.

Münchener Medicinische Wochenschrift. 1896. March.