"EXPERIMENTAL WORK INVOLVED IN THE STUDY AND TREATMENT OF FIVE CASES OF CEREBRO-SPINAL MENINGITIS".

The following work is based on the discovery that in Cerebro-Spinal Meningitis contrary to the usual belief the Cerebro-Spinal Fluid contains a certain small amount of complement.

In all the five cases dealt with complement was found to be present in the Cerebro-Spinal fluid- one provision, however, must be made to this viz:- that the compliment present is markedly diminished, or what is more common, entirely disappears for a variable time after the intrathecal administration of antimeningococcal (M.R.C) serum. This will be demonstrated later in the description of the cases treated.

The details of the experiment designed to show the presence or absence of complement in the Cerebro-Spinal fluid are as follows:-

The Cerebro-Spinal fluid was withdrawn in the usual way, and was always tested both macroscopically, and microscopically for the presence of blood as naturally if the specimen contained blood this would render the experiment fallacious.

Again the Cerebro-Spinal fluid was tested for complement in all cases never less than six hours after its withdrawal from the patient.

Separate experiments were performed on the same fluid by conveying it from the patient to the laboratory,

1. At outdoor temperature in the shade.
2. At 37°C.
3. In a thermos flask surrounded by ice.

No difference was noted in the result as long as the fluid was tested within six hours of its withdrawal. An appreciable diminution of complement was, however, noted after 24 hours at 37°C as would naturally be expected. Thus the fluid as a routine was conveyed at the prevailing atmospheric temperature in the shade, and immediately on its arrival at the laboratory the test was commenced.

It was necessary to prepare a haemolytic couple (hm. cpl.) to act as indicator, and the following was the procedure adopted.

Fresh sheep's blood was caught in a solution of 1.6% citrate of soda in freshly prepared .85% saline solution. Previous to the test the sheep's

corpuscles were washed in .85% saline solution, and centrifuged four times.

A mixture was then made to contain 3.0% washed sheep's blood corpuscles, and five minimum haemolytic doses of haemolysin (i.e. rabbit's serum which has been sensitised against sheep's blood) in freshly prepared .85% saline solution.

It is to be understood that in all the succeeding experiments the haemolytic couple will always be prepared as above described.

Some of the Cerebro-Spinal fluid to be tested was put in the water bath at 55°C for half an hour to inactivate, and then it was used as a control.

For the experiment proper twelve agglutination tubes were used in a Wassermann bath tray- five were used for the test proper, and seven were used as controls.

The total volume in each tube was always 1.0 c.cm. Into five of the tubes were put the following:-

Tubes.	1.	2.	3.	4.	5.
Cerebro-Spinal fluid.	.1 c.cm.	.2 c.cm.	.25 c.cm.	.5 c.c	.75c.c
Saline .85%	.65c.cm.	.55c.cm.	.5 c.cm.	.25c.c	.0 c.c
Haemolytic Couple.	.25c.cm.	.25 c.cm	.25c.cm	.25c.c	.25c.c

The ingredients were added to the tubes in the following order,

1. Cerebro-Spinal fluid.
2. .85% saline.
3. Haemolytic couple.

Other five tubes were put up in a like manner using, however, in this case the inactivated Cerebro-Spinal fluid in place of the untreated Cerebro-spinal fluid.

Another tube was put up containing .75c.c. of .85% saline solution, and .25 c.c. of haemolytic couple; and yet another tube was usdd containing .1 c.c of any fresh complement containing serum, .65 c.c.. of saline, and .25 c.c.. haemolytic couple. As routine no fresh serum was used whose complement titre was below a dilution of 1 in 20 (i.e. the M.H.D. was either 1 in 20 or higher). This was found out in the usual way for complement titration as in the Wassermann reaction.

The last seven tubes acted as controls.

The whole series of tubes were then put in the water bath at 37° C for half an hour being, however, shaken every ten minutes.

The results were read off as soon as the corpuscles had had time to settle, and again after standing 18 hours at room temperature.

If the test is correct there should be no haemolysis in any of the tubes containing inactivated Cerebro-Spinal fluid nor in the saline control tube.

There, however, should be complete haemolysis in the tube containing serum with free complement.

Any test in which the controls were not satisfactory was discarded, and it can therefore be assumed that the controls were correct in all the experiments here recorded.

In order to read the results somewhat similar symbols to those used in the Wassermann test are employed, viz,

+ = no haemolysis.

± = faint haemolysis

≡ = moderate "

± with ≡ = nearly complete haemolysis.

− = complete haemolysis.

A typical experiment will now be shown in tabular form with the symbolic readings attached.

Tubes.	1	2	3	4	5	6	7	8	9	10	11	12.
Cerebro-Spinal fluid unheated.	·1 cc.	·2 cc.	·25 cc	·5 cc	·75 cc	−	−	−	−	−	−	−
Cerebro-Spinal fluid heated.	−	−	−	−	−	·1 cc.	·2 cc.	·25 cc.	·5 cc.	·75 cc	−	−
.85% saline.	·65 cc.	·55 cc	·5 cc.	·25 cc	−	·65 cc	·55 cc	·5 cc	·25 cc	−	·75 cc	·65 cc.
Serum containing complement.	−	−	−	−	−	−	−	−	−	−	−	·1 cc.
Haemolytic couple.	·25 cc.	·25 cc	·25 cc.	·25 cc	·25 cc	·25 cc	·25 cc	·25 cc	·25 cc	·25 cc	·25 cc	·25 cc.
Results.	+	±	± ≡	± ≡	± ≡	+	+	+	+	+	+	−

The following table contains a list of results of all the tests performed.

RESULTS.

Case.	Date.	Tube 1.	Tube2.	Tube 3.	Tube 4.	Tube 5.
1	1st day.	+	+	+	±	±
1	2nd day.	+	+	+	+	+
2	3/2/19.	+	±	±	±	±
2	4/2/19.	+	+	+	+	+
2	5/2/19.	+	+	+	+	+
2	6/2/19.	+	+	+	+	+
2	7/2/19.	+	+	+	+	+
2	9/2/19.	+	+	+	+	+
2	11/2/19.	+	+	+	+	+
2	13/2/19.	+	+	+	+	+
2	16/2/19.	+	+	+	+	+
2	19/2/19.	+	+	+	+	±
3	17/12/18.	+	±	±	±	−
3	19/12/18.	+	+	+	+	+
3	21/12/18.	+	+	+	±	±
3	28/12/18.	+	±	±	±	±
3	29/12/18.	+	+	+	+	+
4	20/12/18.	+	+	+	+	±
4	24/12/18.	+	+	+	+	±
4	28/12/18.	+	+	+	±	±
5	4/12/18.	+	±	±	±	±
5	6/12/18.	+	±	±	±	±
5	10/12/18.	+	±	±	±	±
5	31/12/18.	±	±	±	−	−
5	2/1/19.	±	±	±	±	±
5	21/1/19.	+	±	±	±	±
5	22/1/19.	+	+	+	±	±

The next point noted was that after giving the antimeningococcal serum (M.R.C.) intrathecally the complement was either markedly diminished or disappeared entirely for a variable time. This meant that the complement was either used up in complement deviation or in complement fixation. It was also noted that after the cessation of the intrathecal administration of the antiserum the complement reappeared in the Cerebro-Spinal fluid after a variable interval. The amount of Complement present, and the rapidity of its reappearance after the cessation of the intrathecal medication appear to depend on two factors-

1. Acuteness of the meningeal lesion.
2. Extent of lesion.

These factors were arrived at by contrasting the amount of complement present in mild, and severe cases, and also by the Post-Mortem findings in one case.

Naturally as the cases dealt with are so few too much reliance should not be placed on the last statement until it is further corroborated by more cases.

The first question to study is how much complement in the Cerebro-Spinal fluid of a case of Cerebro-Spinal Meningitis is deviated by an ordinary intrathecal dose of M.R.C. antimeningococcal serum.

Taking the maximum amount of complement present in any of the tests made I find that it was in the Cerebro-Spinal fluid of case "S" (Class 2 Case 5 Page 37) tested on 31/12/18. Here there was just complete haemolysis in tube 4 i.e. in .5 c.c. of Cerebro-Spinal fluid. (Note that the total volume used was 1.0 c.c., and of that there was .25 c.c. of haemolytic couple, thus being truly comparable to the under mentioned experiment).

EXPERIMENT. The complement in fresh guinea pig's serum was titrated as follows;- .25 c.c. of varying dilutions of guinea pig's serum was put up with .5 c.c. of .85% saline solution, and .25c.c. of the haemolytic couple.

This was put in the water bath for half an hour at 37°C, being shaken every ten minutes.. The results were read off- the minimum haemolytic dose being the highest dilution of guinea pig's serum which gave complete haemolysis e.g. in one such experiment which shall now be described there was complete haemolysis up to, and including, the tube containing a dilution of 1 in 50 of guinea pig's serum. Therefore the complement present in .5 c.c. of Cerebro- Spinal fluid + .25 c.c. saline = complement present in .25 c.c. of guinea pig's serum diluted 1 in 50 + .5 c.c. saline.

Tubes were now put up containing .25 c.c. of a 1 in 50 dilution of guinea pig serum + .25 c.c. of varying dilutions of antiserum (type 1 was used in this case) viz, pure, 1 in 2, 1 in 3, 1 in 4, 1 in 5, 1 in 10, and so on to 1 in 100 + .25c.c. of a .85% saline solution + .25cc haemolytic couple. The tubes were immediately put in the water bath at 37°C for half an hour, thus imitating as far as possible in vitro what happens when the antiserum is given intrathecally.

After half an hour the tubes were removed, and the results read both immediately after the corpuscles had settled, and also after standing for 18 hours at room temperature.

This described experiment was done in duplicate, and it was found that there was no haemolysis present up to, and including the tube containing a 1 in 4 dilution of the type 1 antimeningococcal serum. In these experiments a normal saline control is absolutely essential for comparative purposes.

Thus:- .25 c.c. of a 1 in 4 dilution contains $\frac{1}{4}$ X $\frac{1}{4}$c.c of pure antiserum = $\frac{1}{16}$cc. of pure antiserum.

But the complement deviated by this = complement in .25 c.cs. of a 1 in 50 dilution of guinea pig serum,
= complement in .5 c.cs of Cerebro-Spinal fluid.

Therefore the complement deviated by $\frac{1}{16}$ c.c of antiserum = complement in $\frac{1}{2}$ c.c of Cerebro-Spinal fluid.
That is the complement deviated by 30 c.cs of antiserum = complement in 240 c.cs of Cerebro-Spinal fluid. 30 c.cs. of antiserum is taken as it is the usual intrathecal dose of antimeningococcal serum where over 60 c.cs. of Cerebro-Spinal fluid have been withdrawn.

As 60 c.cs. of Cerebro-Spinal fluid is a good average amount to be withdrawn at one lumbar puncture i.e. until the fluid comes out just under the normal pressure, there is rarely likely to be more than 60 c.cs left in the Cerebro-Spinal system (i.e. unless the case has developed hydrocephalus).

This gives one a striking idea of the large amount of Cerebro-Spinal fluid rendered inert as far as complement is concerned by the intrathecal administration of antimeningococcal serum (M.R.C.) to a case of Cerebro-Spinal Meningitis.

This amount is rendered inert purely by means of the complement deviating properties of the antiserum without taking into account its complement fixing capabilities.

(N.B. In one case on which a Post-Mortem was performed soon after death it was calculated that the Cerebro-Spinal system contained about 100.0 c.cs of Cerebro-Spinal fluid. This case showed a commencing hydrocephalus).

This marked complement deviating property of the M.R.C. antimeningococcal serum together with the fact that the Cerebro-Spinal fluid in a case of Cerebro-Spinal Meningitis contains very little complement appears to be a point against the intrathecal medication especially as one is recommended to give it daily for at least four days no matter what happens.

If the results of the complement tests be followed in all the five cases described it will be noticed that, having given the antiserum, the complement disappears, or diminishes for a variable time afterwards. This time, as above stated, appears to vary with the activity, and extent of the meningeal inflammatory lesion, and also with the amount of antiserum given.

If the inflammatory lesion is very active the complement may reappear within 24 hours after giving a normal dose of antiserum intrathecally, say 20 to 30 c.cs; but in other cases it appears to take longer- it may be three or four days. Here lies the difficulty. If the antiserum be given in such an amount, and frequency that no free complement is ever found in the Cerebro-Spinal fluid it follows that the complement binding antibodies are not made full use of.

The next point that arises is as to how one is to get over this complement deviating property of the antiserum. This appears to be possible as follows:-

1. By diminishing the dose.
(a). By giving a smaller dose frequently.
(b). " " " larger dose (20 to 30 c.cs.) every three or four days.

Here one would have to suit the dose to the severity of the lesion i.e. an active lesion would produce more complement, and would thus stand a bigger dose intrathecally.

In any case the dosage would have to be much smaller than it is at present the habit to administer, and as one believes in giving large doses of antiserum it is necessary to look for other means of combating this difficulty.

Whilst concentrating ones attention on the complement fixing properties of the antiserum one is apt to forget that it is only one amongst

others e.g. opsonic effect etc.,of the beneficial factors found in the antiserum, and one should remember that the diminution in the amount of antiserum given also diminishes the dose of these other beneficial properties.

2. Another method strikes one as feasible, and that is to give the antiserum free of its complement deviating properties. This can be done in two ways.

a. To give the fresh horse antiserum i.e. containing free complement which is not practicable in most cases.
b. To give the antiserum along with a sufficiency of complement containing serum e.g. human,in order to get rid of its complement deviating properties.

This latter method (2b) was tried on two cases-one moribund- in which the lumbar puncturing,and intrathecal medication of M.R.C. antimeningococcal serum had failed. It appeared to have neither a beneficial,nor the reverse effect.

A detailed account of the experiments involved with a probable explanation of the failure will now be given.

The first thing was to find out how much serum containing complement should be given to neutralise the amount of antiserum administered. As my own blood was used its serum was first titrated to find out the minimum haemolytic dose. This was done as previously described,and it was found that the blood serum showed a titre of 1 in 20.

Next a series of tubes were put up containing gradually increasing amounts of antiserum (from .1 c.c. to 1.2c.c increasing by .1 of a c.c.) To each tube was added .2 c.cs. of my own serum+.5 c.cs. haemolytic couple,and the whole was made up to a total volume of 2.0 c.cs. by adding the appropriate amount of .85% freshly prepared saline solution. The method of adding the ingredients was as follows- First the antiserum ,then my own serum, and then the saline were put in the tubes. This was left for half an hour at room temperature, and then put in the waterbath at 37° C for half an hour- thus imitating the procedure of giving the mixed sera to the patient.

The haemolytic couple was then added, and the tubes were put back in the waterbath for another half hour at 37°C, but were shaken every ten minutes. After removing the tubes from the waterbath the results were read off immediately the corpuscles had settled (this could be done quickly by centrifugalising the tubes), and the results corroborated after standing for eighteen hours at room temperature. A normal saline control

control is again essential for comparative purposes.

In one such experiment the tube containing .7 c.cs. of antiserum showed very faint haemolysis whilst the tube containing .8 c.cs. of antiserum showed no haemolysis.

Thus .2 c.cs. of my own serum contained just enough complement to neutralise the complement deviation power of .7 c.cs. of the antiserum. Therefore one should use at least 1.0 c.c of my own serum with 3.5 .c.cs of antiserum.

This result could be arrived at indirectly in another way by a slight modification of the experiment which dealt with the amount of complement deviated in the Cerebro-Spinal fluid in a case of Cerebro-Spinal Meningitis(vide ante pp.5 and 6)

Instead of adding the haemolytic couple at once the other ingredients were first kept at room temperature for half an hour, and then in the waterbath at 37°C for half an hour before the haemolytic couple was added. After this addition the tubes (being shaken every ten minutes) were again kept in the waterbath at 37°C for another half hour, and the results were then read off immediately the corpuscles had had time to settle.

The rest of the experiment was carried out in a similar manner to the one described previously. The result was slightly different as it was found that no haemolysis was present up to and including the tube containing a dilution of 1 in 7 of the antiserum(The previous experiment without the above modifications gave a reading of 1 in 4).

Now as the M.H.D. of the guinea pig serum was 1 in 50 and as the M.H.D. of my own serum was 1 in 20,

Therefore the complement in .25 c.c. of a 1 in 50 dilution of guinea pig serum = complement in .25c.c. of a 1 in 20 dilution of my own serum.

Therefore the complement in .25 c.cs of a 1 in 20 dilution of my own serum was neutralised by the complement deviation properties of .25 c.c. of a 1 in 7 dilution of the antiserum i.e. $\frac{1}{4} \times \frac{1}{20}$ c.c of my own serum should be used with $\frac{1}{4} \times \frac{1}{7}$ c.c. of the antiserum, i.e. $\frac{1}{80}$ c.c. of my own serum should be used with $\frac{1}{28}$ c.c. of antiserum, i.e. roughly 1 c.c. of my own serum should be used with 3 c.cs. of antiserum. By the direct method the ratio was 1 to $3\frac{1}{2}$, and not 1 to 3 as found by this indirect method.

To obtain my own serum it was necessary

to withdraw a sufficiency of blood from the median basilic vein, and then pipette off the serum under aseptic precautions into the ampoule containing the proposed dose of antimeningococcal serum. "10"

Thus 10 c.cs. of my own serum were given with 35 c.cms. of antiserum in one case, and with only 25 c.cs. of antiserum in the other case. No beneficial effect was noticed though it should be noted that both cases were seriously ill, and had failed to show any previous good effects from lumbar puncturing, and the intrathecal administration of the antiserum.

In order to corroborate the experimental work the mixtures of my own serum and the thera peutic antimeningococcal serum were tested for the presence of free complement in the usual way i.e. .75 c.c. of each mixture was put up with .25 c.c. of haemolytic couple. After thirty minutes in the waterbath at 37°C the tubes were centrifugalised, and then the results read off.

In the case where 35 c.cs. of antiserum were given with 10 c.cs. of my own serum (M.H.D.=1 in 20) very faint haemolysis (±) was noticed.

In the other case where only 25 c.cs. of antiserum were given with 10 c.cs. of my own serum (M H.D.= 1 in 20) moderate haemolysis (±) was present.

This confirms the experimental work above described.

Now it was noticed that in spite of giving sufficient fresh serum with the therapeutic antiserum so as to obviate the complement deviating properties of the antiserum the Cerebro- Spinal fluid withdrawn from one of the cases on the day following the administration (Class 2 Case 3 date 29/12/18) contained no complement. Does this mean that the complement has been used up entirely in complement fixation, or is it possible that the complement deviating properties of the antiserum have returned?. No doubt on administration a certain amount of complement is used up in fixation, but as time lapses complement also deteriorates at a temperature of 37°C, and if this be so do the complement deviating properties return?.

To try and settle this point a further series of tests were performed.

5 c.cs. of antiserum were mixed with 20 c.cs. of saline solution , and part of this was filtered through a Doulton filter candle.

Unfiltered = "A": Filtrate = "A1".

5 c.cs. of the same antiserum were

mixed with 20 c.cs. of saline solution which contained sufficient fresh guinea pig serum to produce excess of complement.

Half of this was filtered through a Doulton filter candle. Now both unfiltered and filtrate were divided into two parts.

One part of each was kept in the incubator (37°C) for two days until it was found that no free complement could be demonstrated by the usual test.,

Unfiltered = "B" Filtrate = "B1".

The remaining part of each was inactivated at 55°C for half an hour in the waterbath.

These were called respectively

Unfiltered = "C" Filtered = "C1".

All these were now subjected to the test for complement deviation.

.25c.c.of each was put up with .25 c.c.of varying dilutions of guinea pig serum,and .25c.c.of saline solution. This was kept for half an hour at room temperature,and for another half hour at 37°C in the waterbath before .25 c.c. of the haemolytic couple was added.

The tubes were again kept for another half an hour in the waterbath at 37°C, being shaken every ten minutes, before the results were read.

The tube containing the highest dilution of guinea pig serum compatible with complete haemolysis was read off.

RESULTS:-

"A" = tube containing a 1 in 5 dilution of guinea pig serum.
"A1"= tube containing a 1 in 10 " " "
pig serum.
"B" = tube containing a 1 in 10 " " "
pig serum.
"B1"= tube containing a 1 in 15 " " "
pig serum.
"C" = tube containing a 1 in 12 " " "
pig serum.
"C1 = tube containing a 1 in 24 " " "
pig serum.

This shows that the complement

deviation properties have returned to a certain extent, and this return is present both in the unfiltered and filtrate.

That these properties are not so great as they were before saturation with complement is shown by comparing the readings of "B" and "C" with "A", and of "B1" and "C1" with "A1".

From this can be deduced the fact that though having once saturated the antiserum with complement it does not mean that its complement deviating properties are entirely satisfied for all time, as no sooner does the free complement disappear than the complement deviating properties commence to return.

This indicates a possible reason for the non-success of the administration of antiserum which is temporarily free of complement deviating properties - this being produced by the addition of fresh serum to the therapeutic antiserum.

Up till now it has been presumed that the little complement there is in the Cerebro-Spinal fluid of a case of Cerebro-Spinal Meningitis is entirely removed by the complement deviating properties of the intrathecally administered antiserum. Is this so or is it used up by complement fixation or perhaps by a combination of deviation and fixation?.

This involves a more complicated experiment.

First it is necessary to imitate as well as possible in vitro what happens in vivo.

One of the largest amounts of complement ever found in the Cerebro-Spinal fluid in a case of Cerebro-Spinal Meningitis was found in that case in which .75 c.c. of Cerebro-Spinal fluid just produced complete haemolysis when mixed with .25 c.c. of haemolytic couple as explained in a previous experiment.

Now taking 120 c.cs. as the probable maximum of fluid left in the Cerebro-Spinal system after lumbar puncture of an ordinary case of Cerebro-Spinal Meningitis (i.e. without hydrocephalus) the amount of antiserum given intrathecally would in all likelihood be 30 c.cs.

Now find out the M.H.D. of some fresh guinea pig's serum as previously described i.e. .25 c.c. of varying dilutions of guinea pig's serum + .5 c.c saline + .25c.c. haemolytic couple were placed in the waterbath at 37°C for half an hour, the tubes being shaken every ten minutes.

The M.H.D. was taken as the tube containing the highest dilution of guinea pig's serum compatible with complete haemolysis.

In this way an imitation of the Cerebro-Spinal fluid can be obtained in as far as its complement content is concerned.
Thus .75 c.c. of Cerebro-Spinal fluid contains as much complement as .25c.c. of guinea pig's serum (diluted so that .25c.c. contains 1 M.H.D. of complement) + .5c.c. saline.
Therefore 120 c.cs of Cerebro-Spinal fluid contains as much complement, and also complement in the same strength c.c. for c.c. as 40 c.cs. of guinea pig's serum diluted as above described + 80 c.cs. saline solution.

This mixture is to be known as mixture "A".

This mixture, however, does not contain any meningococci. To imitate this it is necessary to make up an emulsion of meningococci of a strength of about 2000 millions per 1.0 c.c. As this emulsion deviates complement it is again necessary to titrate the guinea pig's serum in its presence in order to get the M.H.D. properly adjusted.

Thus we mix .25 c.c. of varying dilutions of guinea pig's serum with .25 c.cs. of the emulsion, and .25c.c. saline solution. This is kept for half an hour at room temperature, and again for another half an hour at 37°C in the waterbath. .25 c.c. of haemolytic couple is now added, and the tubes, being shaken every ten minutes, are again put in the waterbath for another half an hour at the end of which time the results are read as above described.

Now an imitation of 120 c.cs. of Cerebro-Spinal fluid can be made containing not only a like amount of complement but also meningococci.

.75 c.cs. of Cerebro-Spinal fluid just produces complete haemolysis when mixed with .25 c.cs. of haemolytic couple; also .25 c.cs. of guinea pig serum of such a dilution that when titrated in the presence of the emulsion it was found to contain 1 M.H.D. of complement + .25 c.cs. emulsion + .25c.c. saline solution just produces complete haemolysis with .25c.cs. haemolytic couple.
Therefore 120 c.cs. of Cerebro- Spinal fluid is represented by (40 c.cs of guinea pig's serum of above described dilution)
(40 c.cs emulsion
(40c.cs. saline solution.

This mixture is to be known as Mixture "B".

For comparison purposes a control mixture was made up containing 120 c.cs saline.

This mixture is to be known as mixture "C".

Now both mixture "A" and mixture "B" contain an equal amount of free complement.

Heat all three mixtures in the waterbath at 37°C until they reach that temperature. Mixture "B" now imitates as nearly as possible the Cerebro-Spinal fluid in vivo. Mixtures "A" and "C" are used as controls.

Mix thoroughly 100c.cs. of antimeningococcal serum, and bring this to a temperature of 37°C by means of a waterbath. This is now in a similar condition to that that in which one administers it intrathecally to a patient.

Now add 30 c.cs of the antimeningococcal serum (temperature 37°C) to each of the three mixtures (each at 37°C), and after thoroughly shaking each, leave in the waterbath at 37°C for one hour.

After this they are tested as a routine procedure for free complement i.e. .75c.c of each was mixed with .25 c.c of haemolytic couple, and left for half an hour in the waterbath, being well shaken every ten minutes. The tubes are now removed, and centrifugalised . In no case was there any trace of haemolysis thus showing that there was no free complement present.

It is now necessary to filter all three mixtures. The idea is to get rid of all the meningococcci from mixture "B". But why filter the other two mixtures?. The reason is because it was found that mere filtration of the antiserum produced a diminution in its complement deviating properties.

In order to eliminate this fallacy all the mixtures were filtered through filter candles of as like a like a porosity as possible(see later). Each fluid was filtered a like number of times (3), and this was done under as nearly equal conditions as far as pressure, and time of filtration were concerned.

Regarding this diminution of complement deviating properties produced by filtration the following experiment was done to illustrate it. 5 c.cs of antimeningococcal serum were diluted with 20 c.cs. of normal saline solution. Part of this was filtered through a Doulton candle, and then both the unfiltered portion, and the filtrate were tested for their complement deviating properties.

Thus .25 c.c. of both the unfiltered, and the filtrate were put up with .25c.c of varying dilutions of fresh guinea pig's serum, and .25 c.c normal saline (.85%) solution. The tubes were now allo

allowed to stand at room temperature for half an hour, and then in the waterbath at 37°C for half an hour. .25 c.c. of haemolytic couple was now added, and the tubes, being shaken every ten minutes, were again left in the waterbath for half an hour at 37°C.

The highest dilution of guinea pig's serum which did not prevent complete haemolysis was then read off in both series.

Unfiltered = 1 in 5 dilution of guinea pig's serum.
Filtrate = 1 in 10 " " " " "

This shows that filtration alone removes a certain amount of the deviating properties of the therapeutic antimeningococcal serum.

The reason of the filtration was primarily as stated above to get rid of the meningococci, and then to test the filtrates for first complement deviation, and then complement fixation.

By this means it is intended to find out how the complement is used up.

If filtrate "A1" deviates less complement than filtrate "B1" and fixes more complement than "B1" then we have a double proof that some of the complement in mixture "B" has been used up in complement fixation. Whereas if the complement deviating and fixing properties of both filtrates are the same then it follows that all the complement in mixture "B" (allowance having already been made for the complement deviating properties of the emulsion) has been used up in deviating, and none in fixing. The latter is what was found.

Now to consider the experiment proper.
The following was the composition of the mixtures:-
The M.H.D. of the guinea pig's serum was 1 in 60.
" " " " " " " in the presence of .25 c.c. of emulsion was 1 in 35.

Mixture "A" (30 c.cs. of antiserum (type 1)
(40 " " 1 in 60 dilution of guinea pig's serum.
(80 " " .85% saline solution.

Filtrate "A1" = Mixture "A" filtered.

Mixture "B" (30 c.cs. of antiserum (type 1)
(40 " " emulsion " "
(40 c.cs. " 1 in 35 dilution of guinea pig's serum.
(40 c.cs. of saline (.85%) solution.

Filtrate "B1" = Mixture "B" filtered.

Mixture "C" (30 c.cs. of antiserum.
(120 " " .85% saline solution.

Filtrate "C1" = Mixture "C" filtered.

A complement deviation experiment was performed on mixtures "A" and "C" and filtrates "A1", "B1, and "C1". It was not done on mixture "B" on account of the fallacy which would be introduced by the presence of the meningococci.

.25 c.c. of varying dilutions of fresh guinea pig's serum (from pure up to 1 in 60) were mixed with .25 c.c. of saline, and .25 c.c. of the above mentioned mixtures, and filtrates. These tubes were kept at room temperature for half an hour, and again for half an hour in the waterbath at 37°C.° .25 c.c. of haemolytic couple was now added, and shaking the tubes every ten minutes, they were subjected to a temperature of 37°C in the waterbath for another half an hour.

The tube containing the highest dilution of guinea pig's serum compatible with complete haemolysis is read off thus.

Mixture "A" = 1 in 20 dilution of guinea pig's serum.
Filtrate "A1" = 1 in 25 " " " " "
Filtrate "B1" = 1 in 25 " " " " "
Mixture "C" = 1 in 10 " " " " "
Filtrate "C1" = 1 in 15 " " " " "

From this we gather,

1. That filtration diminishes the complement deviating properties of the antiserum-vide greater deviation in mixtures "A" and "C" when compared with filtrates "A1" and "C1" respectively.

2. That the addition of complement diminishes the complement deviating properties of the antiserum, and also that this diminution can be shown in this experiment- vide greater deviation in the control mixture "C" as compared with mixture "A", and also greater deviation in the filtrate "C1" as compared with filtrates "A1" and "B1". This means that as far as the deviation part of this experiment is concerned it forms a pretty accurate guide as to how the complement is used up.

3. As an equal amount of complement deviation is shown in filtrates "A1" and "B1" one naturally concludes that an equal amount of complement has been used up in deviation in the original mixtures "A" and "B" which means that none of the complement in mixture "B" has been used up for fixation purposes.

Now to corroborate this one turns to the complement fixation properties of the mixtures, and filtrates.

The mixtures, and filtrates were now diluted 1 in 5 for convenience (.25 c.c. of the undiluted completely prevented haemolysis with 3 M.H.D. of guinea pig's serum).

3 M.H.D. of guinea pig's serum was chosen as the standard dose of complement. An emulsion of meningococci about 2000 millions per 1.0 c.c. was made (type 1 in this case), and .25 c.c. was titrated with 3 M.H.D. of guinea pig's serum to see that it did not prevent complete haemolysis.

It was done as follows:-

.25 c.c. of the emulsion was mixed with .25 c.c. of 3 M.H.D. of guinea pig's serum, and .25 c.c. of saline solution. This was well mixed, and left at room temperature for half an hour, and then at 37°C in the waterbath for half an hour. .25 c.c. of haemolytic couple being added the tube was left for half an hour in the waterbath at 37°C, shaking every ten minutes.

The emulsion was only used when complete haemolysis occurred in this tube.

Now .25 c.c. of each specimen (diluted 1 in 5) was mixed in varying dilutions (pure to 1 in 80) with .25 c.c. of the emulsion, and .25 c.c. of 3 M.H.D. of guinea pig's serum (naturally the guinea pig's serum was titrated at the commencement of the experiment. In this case 1 M.H.D. = 1 in 60. Therefore 3 M.H.D. = 1 in 20). The tubes were left for half an hour at room temperature, and then for half an hour in the waterbath at 37°C. The haemolytic couple was now added in .25 c.c. quantities, and, being shaken every ten minutes, the tubes were left for another half an hour in the waterbath at 37°C. The lowest dilution of the filtrates, and mixtures which did not prevent complete haemolysis was then read off.

Thus,	Mixture "A"	= dilution of	1 in 80.
	Filtrate "A1"	= " "	1 in 50.
	Filtrate "B1"	= " "	1 in 50
	Mixture "C"	= " "	1 in 120.
	Filtrate "C1"	= " "	1 in 80.

Now both filtrates of mixture "A" and "B" i.e. filtrates "A1" and "B1" fix the same amounts of complement in similar dilution (1 in 50), thus showing that the complement was not used up in fixing antibodies in the original mixture "B" otherwise one would have expected the filtrate "B1" to fix an equal amount of complement only in a lower dilution than in the case of filtrate "A1", which was not so.

We notice that filtrate "C1" fixes complement

complement in a higher dilution(i.e.weaker) than in the case of filtrates "A1"and "B1". The same can be said about mixture "C" when compared with mixture "A".

This appears to show that the treating of the antiserum with complement does of itself lower the complement fixing powers of the antiserum; but as the complement deviating powers of .25 c.c. of the filtrate "C1" were much higher than those of .25 c.c. of either filtrates "A1" or "B1".(which latter two were the same vide ante).this will account for the apparent though not real higher complement fixing powers of the filtrate "C1".

The same can be said when we compare mixture"C" with mixture "A" as regards their complement fixing properties.

As control of this experiment it is necessary necessary to prove that the filtrate from the emulsion of the meningococci neither deviates nor fixes complement.

40 c.cs of the emulsion were mixed with 110 c.cs of saline and filtered. The filtrate was now tested as follows,

1. For complement deviation.

Some guinea pig's serum was first titrated as regards its complement content in the usual way.

The result was that the M.H.D. = 1 in 60.

Now .5 c.c. of the filtrate was mixed with .25 c.c. of a dilution of 1 in 60 of guinea pig's serum (i.e. 1 M.H.D.), and left for half an hour at room temperature, and then for another half an hour at 37°C in the waterbath. .25 c.c of haemolytic couple was now added, and the tube again put in the waterbath for another half an hour at 37°C , being well shaken every ten minutes.

On removal the contents of the tube showed complete haemolysis which proves that the filtrate of the emulsion does not deviate complement.

2. For complement fixation.

An emulsion(weak) of the meningococcus is made,and titrated with 1 M.H.D. of guinea pig's serum as follows:-

Varying amounts of emulsion (.1; .25;.3 .4,.5,c.c.) were mixed with sufficient saline solution(.85%) in each case to bring the total fluid in each tube to .5c.c.(i.e. .4: .25: .2: .1: 0.0 c.c. of saline solution respectively). .25 c.c of a 1 in 60 dilution of guinea pig's serum (i.e. 1 M.H.D.) was then added to each tube.

The tubes were allowed to stand for half an hour at room temperature, and for another half an hour at 37°C in the waterbath, and then .25 c.c haemolytic couple was added. The tubes, being shaken every ten minutes, were again left for another half an hour at 37°C in the waterbath.

The highest amount of emulsion which did not prevent complete haemolysis was then read off. In this case it was .25 c.c.

Now in the test proper .25 c.c. of the filtrate was mixed with .25 c.c. of the emulsion, and with .25 c.c. of a 1 in 60 dilution of guinea pig's serum. (1 M.H.D.)

The tube was left for half an hour at room temperature, and again for another half an hour at 37°C in the waterbath. .25 c.c. of haemolytic couple was then added, and the tube was left for another half an hour at 37°C in the waterbath taking care to shake it well every ten minutes. On reading the result it was found that this tube was completely haemolysed.

This proves that the filtrate contains no complement fixing antibodies.

One was able to prove that the experiment as far as the complement deviating property of the antiserum was concerned was fine enough, and accurate enough to demonstrate the points necessary vide p(p.16) Is this the case with the complement fixing properties or is the amount of complement fixing antibodies which might possibly be used up so small as compared with the large total amount in the mixture "B" that the test is not fine enough to show the relatively minute possible difference?. Again the possibility strikes one of the emulsion not being strong enough to fix sufficient complement fixing antibodies in order to show a difference in this test. A further series of experiments were done using however 5c.cs. of antiserum instead of 30 c.cs. as in the original mixtures "A", "B", and "C", and also both mixtures were saturated with excess of complement i.e. 10c.cs. of fresh guinea pig's serum were added to each. The guinea pig's serum was titrated for its complement content both in the presence of, and also in the absence of emulsion. The M.H.D. of guinea pig's serum = 1 in 60. The M.H.D. of guinea pig's serum in presence of emulsion = 1 in 35. Thus the mixtures were as follows, and were made up as previously described.

Mixture "D" (5.0c.cs antiserum.
(70.0c.cs. saline.
(10.0 c.cs. pure guinea pig's serum.
(40.0 c.cs.(1 in 60) guinea pig's serum.

Filtrate "D1" = mixture "D" filtered.

(70.0c.cs saline + 10.0c.cs of pure guinea pig's serum were used instead of 80.0c.cs. saline as in previous experiment).

Mixture "E" (5.0c.cs. antiserum
(30.0 " saline
(10.0 " pure guinea pig's serum.
(40.0 " emulsion.
(40.00" (1 in 35) guinea pig's serum.

Filtrate "E1" = mixture "E" filtered.
(30.0c.cs saline + 10.0c.cs of pure guinea pig's serum were used instead of 40.0c.cs saline as in previous experiment).

Mixtures "D" and "E" and filtrates "D1" and "E1" were all tested for presence of complement in the usual way, and it was found that .75 c.cs of each produced complete haemolysis when mixed with .25 c.c. of haemolytic couple(The Doulton filters did not much influence the passage of complement into the filtrates). This shows that there was free complement in all. Before doing the test proper all these were inactivated as far as complement was concerned by keeping them at 55°C in the waterbath for half an hour, and, as a control of this, each was again tested for presence of complement as above described. No complement was found i.e. there was no haemolysis in any of the tubes.

Again on account of the small amount of antiserum used it was not found necessary to dilute the filtrates before performing the complement tests. With the above modifications the experiment was carried out in exactly the same way as described for mixtures "A", "B" and "C" and filtrates "A1", "B1" and "C1"

The results were,

1. For complement deviation.

Filtrate "D1" = complete haemolysis up to a dilution 1 in 50.
Filtrate "E1" = complete haemolysis up to a dilution 1 in 50.

2. For complement fixation.

Filtrate "D1" = complete haemolysis down to a dilution of 1 in 20.
Filtrate "E1" = complete haemolysis down to a dilution of 1 in 20.

Now in this case one would have expected that in the complement fixation the filtrate "E1" would have fixed less complement than in the case of filtrate "D1" i.e. would have required a lower

dilution (stronger mixture) to fix the same amount of complement as in the case of filtrate "D1". In other words one would have expected to see a difference because in mixture "E" a certain amount of complement fixing antibodies must have been fixed, and thus have left a diminished amount in filtrate "E1". The complement deviation part of this experiment is of course no guide as both mixtures have been treated with excess of complement. It, however, shows that this excess of complement has reduced them to the same condition as far as their remaining complement deviating properties are concerned—thus making the mixtures truly comparable before the complement fixation test is performed.

This experiment was again repeated in a similar manner using 2.0 c.cs. of antiserum instead of 5.0 c.cs.

The results were,

1. Complement deviation
   Filtrate "F1" = 1 in 50
   Filtrate "G1" = 1 in 50.

2. Complement fixation.
   Filtrate "F1" = 1 in 20.
   Filtrate "G1" = 1 in 15.

This at last shows that in the original mixture "G" a certain amount of complement fixing antibodies has been used up.

It also demonstrates that for our purposes the complement fixation part of the experiment is not of sufficient fineness. That this experiment is all right when the difference between the large amount of complement fixing antibodies still left, and the relatively small amount used up is not so great will now be shown.

In this case not only is excess of complement used, but also a very strong meningococcal emulsion. The mixtures were as follows,

Mixture "H" (2.0c.cs. antiserum.
(100.0c.cs saline.
(20.0c.cs. of pure guinea pig's serum.

Filtrate "H1" = mixture "H" filtered.

Mixture "K" (2.0 c.cs. antiserum.
(40.0c. cs. very strong emulsion.
(20.0c.cs of pure guinea pig's serum.
(60.0c.cs. saline.

Filtrate "K1" = mixture "K" filtered.

The results were.

1. Complement deviation.

Filtrate "H1" = 1 in 30.
Filtrate "K1" = 1 in 30.

2. Complement fixation.

Filtrate "H1" = 1 in 15.
Filtrate "K1" = no fixation(haemolysis in all tubes).

This shows that in mixture "K1" all the complement fixing antibodies have been used up, and none left in the Filtrate "K1".

On performing the test again using a slightly different strength of emulsion, and also titre of guinea pig's serum "K1" showed incomplete haemolysis in the tube containing the pure filtrate thus,

1. Complement deviation.

Filtrate "H1" = 1 in 25.
Filtrate "K1" = 1 in 25.

2. Complement fixation.

Filtrate "H1" = 1 in 15.
Filtrate "K1" = 1 in 1 (i.e. pure filtrate "K1")

This merely illustrates the well known fact that for a complete reaction to occur between antibody, antigen, and complement a definite amount of each must be present. If these amounts be not correct the reaction is imperfect as is shown by this last experiment.

Before closing the description of the experimental work it might be of advantage to describe a few interesting points regarding the use of the filter candles. As regards these, Doulton's were used but caused a great deal of trouble as it was found that in a large number the porcelain candle was loose in its socket, and in others the cement fitting was not firm but leaked badly. As numerous experiments were originally spoilt by this fault it was necessary to thoroughly test each filter before use. The great difficulty was in properly grading the porosity of the filter. The usual method of testing by means of varying sizes of organisms is a very crude, and tedious method.

It may be of interest to note that from the above noticed fact that filtration varies the complement deviation properties of a serum another means of grading the porosity of a filter is

indicated which would probably be both quicker, and more accurate.

One now looks around to see if the results obtained from the experimental work can be made to subserve any useful purpose. Obviously as far as complement fixing antibodies are concerned the administration of antimeningococcal serum (M.R.C) intrathecally not only does no good, but does harm. The giving of it with fresh complement containing serum so as to obviate its complement deviating properties does not appear to be successful—A possible reason for this having been shown experimentally.

A more rational as well as scientifically accurate method appears to suggest itself as likely to be more succesful.

It is proposed to administer large doses intravenously, and to keep up the effect by either intravenous, intramuscular, or subcutaneous administrations, thereafter. By this means the antibodies etc. are introduced into the system.

Next it is necessary to get them to the site of the lesion. If the case be an acute septicaemic one this is immediately effected. If, however, it be the ordinary meningeal lesion a flow of serum must be instituted into the Cerebro-Spinal system as it is done in Nature's effort to effect a cure. This can be encouraged by means of frequent lumbar puncturing, and drainage of the Cerebro- Spinal system following the well known surgical treatment of "Where there is pus let it out". If, however, the flow of serum be not sufficient to meet the needs of the case recourse might be had to Sir A Wright's method of hypertonic saline treatment.

The next question that arises is as to whether hypertonic saline given intrathecally would produce any injurious effects on the central nervous system. To find this out a few experiments were performed on rabbits..

In each case by means of lumbar puncture 4c.cs. of sterile hypertonic (10%) saline solution were introduced into the Cerebro-Spinal system.

In none of these cases were any ill effects noticed, and post-mortem, from two to three days later, showed nothing abnormal.

A few more experiments were done on other rabbits regarding the effect of intravenous administration of antimeningococcal serum. Nothing new was found out regarding its administration but the following well known points might be emphasied.

To escape ill effects give the antiserum

at as nearly the body temperature as possible i.e. as it escapes from the needle, and not as it is in the resevoir. Also give it slowly, and at a low pressure, and lastly keep the animal warm not only during, but also after the operation.

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CASES.

As a preliminary note it may be mentioned that a detailed clinical account of the cases is not given other than that part which has direct bearing on the experimental work involved.

With the exception of case one the others were treated by the Doctor in charge of the Isolation Hospital at which the patient was kept- the bacteriologist only being called in for diagnosis, and advising of treatment, and in some cases to assist in the operation of lumbar puncture, and intrathecal administration of antimeningococcal serum.

Of the five cases of Cerebro-Spinal Meningitis with which this article deals the striking feature was that those cases, which were early diagnosed and treated, usually got better; whilst those, which were for a long time undiagnosed and thus untreated for Cerebro-Spinal Meningitis, usually died. This is the common experience of all who treat their cases by means of the frequent withdrawal of the Cerebro-Spinal fluid, with or without other therapeutic measures e.g. intrathecal or intravenous administration of antimeningococcal serum, vaccines, etc. This statement must of course be modified in cases of the the fulminant variety where the patient dies in from 12 to 48 hours.

As will be gathered from a description of the cases intrathecal administration of the M.R.C. antimeningococcal serum was most disappointing, and in some cases appeared to aggravate the patient's condition. The one treatment which appeared always to do good was the operation of lumbar puncture with the withdrawal of Cerebro-Spinal fluid. The amelioration of the patient's condition by this method of treatment alone often yields most striking results. This latter has been noted in previous epidemics.

I think the general diminution of the mortality in Cerebro-Spinal Meningitis is probably largely due to the more rapid diagnosis, and early drainage of the Cerebro-Spinal system by means of lumbar puncture. Other therapeutic measures appear to be of little, and in some cases of very doubtful value.

In describing the cases it is proposed to introduce a few notes at the end of each case dealing with the salient features.

These cases naturally fall into two classes viz, "A" = early diagnosed- under one week- two in number: "B" = Late diagnosed- over one week- three in number.

As it turns out this classification coincides with another for the five cases in question viz.

"A" = recoveries = two in number.

"B" = deaths - three in number.

It is to be understood that the therapeutic antiserum used in all cases was that issued by the Medical Research Committee.

Taking the cases in seriatim we have:-

Class "A".

Case 1. (L) This man was treated under the supervision of my predecessor Lieut: C.Fletcher R.A.M.C.
The chief symptoms were delirium with a temperature of 103°F, relatively slow pulse rate viz 76 per minute, rigidity of the neck and double Kernig (not marked). He had been ill less than a week, and he had a history of vomiting, and of having suffered from a severe headache previous to admission into hospital.
40 c.cs. of Cerebro-Spinal fluid were withdrawn, and 17 c.cs of pooled types 1 and 2 antimeningococcal serum were given intrathecally. The Cerebro-Spinal fluid was turbid, and came out under increased pressure. A type 1 meningococcus was isolated from it.
The complement was present as follows:-

1 = +: 2 = +: 3 = +: 4 = ±: 5 = ±:

To see how much the complement deteriorated on keeping, a further test was done with this specimen of Cerebro-Spinal fluid. The latter was kept for eighteen hours in the incubator at 37°C, and then again tested.
The result was;-

1 = +: 2 = +: 3 = +: 4 = +: 5 = ±:

This shows a slight deterioration of complement as would be expected.
Next day 45c.cs. of Cerebro-Spinal fluid were withdrawn, and it was found to be much clearer. The patient was greatly improved.
30c.cs. of type 1 antimeningococcal serum were given intrathecally.
As the following shows, no complement was present in the Cerebro-Spinal fluid withdrawn.

1 = +: 2 = +: 3 = +: 4 = +: 5 = +:

After this the patient made an uninterrupted recovery.

Notes:- This was a case of moderate severity, early diagnosis, and rapid recovery.
The effect of the lumbar puncturing, and giving of the antiserum intrathecally was good.
A noticeable point is that the antiserum had the effect of depriving the Cerebro-Spinal fluid entirely of its free complement up to at least 24 hours after it had been given.
The amount of complement in the Cerebro-spinal fluid in this case was very small-lesion probably small, and not very active.

Class "A"

Case 2.(Mc. R) This was the only case of the five which I had the opportunity of treating in its entirety.
He was early diagnosed; the first symptoms headache, and stiffness of the neck having commenced four days previous to his diagnosis as Cerebro-Spinal fever. The chief symptoms were stiffness of the neck, headache, malaise, elevated temperature, and double Kernig. There was no vomiting.
The rigidity of the neck, and back afterwards became a most striking feature.
It is interesting to note that he had herpes labialis. No meningococci could be cultivated from the fluid in the blisters.

3/2/19. 30c.cs. of Cerebro-Spinal fluid were withdrawn. It was very turbid, and under marked pressure.
20 c.cs. of pooled 1 and 2 antimeningococcal serum were given intrathecally.
Complement was present in the Cerebro-Spinal fluid as follows:-

1 = +: 2 = ±: 3 = ±: 4 = ±: 5 = ±:

4/2/19. In the agglutination of the organism by the rapid method a type 2 (two) meningococcus was diagnosed; but on awaiting for the proper agglutination test it was found that agglutination occurred with both types 1 and 2 sera up to a dilution of 1 in 400.
The subculture for the absorption of agglutinin test died out in spite of all efforts, and as no further growth from the Cerebro-Spinal fluid could be

obtained, nor could any meningococcus be isolated from the throat, urine, or blood, it was impossible to fix definitely on the type.
It was thus decided to use the pooled types 1 and 2 antisera. One dose of the mono-valent type 2 antiserum was, however, given on the 4/2/19 as a result of the rapid agglutination test.
On this day 55 c.cs. of Cerebro-Spinal fluid were withdrawn, and 20 c.cs. type 2 antiserum were given intrathecally. The Cerebro- Spinal fluid came out under pressure, but was clearer than that withdrawn on the previous day.
As the following shows no complement was present in the Cerebro-Spinal fluid,

1 = + : 2 = + : 3 = + : 4 = + : 5 = + :

5/2/19. Patient i.s.q.
60 c.cs of slightly turbid Cerebro-Spinal fluid were withdrawn, and 30 c.cs of pooled 1 and 2 antiserum were given intrathecally. No complement was present in the Cerebro-Spinal fluid withdrawn.

1 = + : 2 = + : 3 = + : 4 = + : 5 = + :

6/2/19. A marked serum rash was present.
80 c.cs. of Cerebro-Spinal fluid were withdrawn, and 30 c.cs of pooled 1 and 2 antiserum were given intrathecally.
The Cerebro-Spinal fluid showed absence of complement.

1 = + : 2 = + : 3 = + : 4 = + : 5= + :

7/2/19 Patient was if anything a little worse. Rigidity was now marked, and pain in the back and neck was more severe.
60 c.cs. of fairly clear Cerebro-Spinal fluid were withdrawn, and 30 c.cs. of pooled 1 and 2 antiserum were given intrathecally. The test for complement in the Cerebro-Spinal fluid showed its continued absence.

1 = + : 2 = + : 3 = + : 4 = + : 5 = + :

8/2/19 Patient i.s.q.
Decided not to lumbar puncture.

9/2/19 Patient i.s.q. except that the headache was a little worse. 50 c.cs of Cerebro- Spinal fluid were withdrawn under pressure, and 30 c.cs. of pooled 1 and 2 antiserum were given intrathecally. No complement was present in the Cerebro-Spinal fluid withdrawn.

1 = + : 2 = + : 3 = + : 4 = + : 5 = + .

The following night the patient was very restless.
Rigidity was marked, and delirium, and severe pain in the back and neck were present.
A hypodermic of $\frac{1}{4}$ gr of morphia was given.

10/2/19. Patient was a little quieter.

11/2/19. Patient had a good night. Symptoms much the same except that pain was much less, and the delirium had disappeared. Lumbar puncture was again performed, and 50 c.cs. of fairly clear Cerebro-Spinal fluid, under pressure, were withdrawn. 25 c.cs. of pooled 1 and 2 antiserum were administered intrathecally. On the following evening patient again became very noisy, and delirious. Severe headache, and pain in the neck and back were prominent symptoms. The patient got very little sleep in spite of being given $\frac{1}{4}$ gr morphia hypodermically.
No complement was present in the Cerebro-Spinal fluid,

1 = +: 2 = +: 3 = +: 4 = +: 5 = + .

12/2/19. Patient a little better.

13/2/19. 75 c.cs. of Cerebro-Spinal fluid were withdrawn, and 30 c. cs. of pooled 1 and 2 antiserum were given intrathecally.
Complement was still absent from the Cerebro-Spinal fluid.

1 = +: 2 = +: 3 = +: 4 = +: 5 = + .

A few hours after the operation the patient was again much worse, and the prognosis was becoming increasingly grave. A petechial haemorrhagic rash had now appeared on the trunk, and extremities. That evening a $\frac{1}{4}$ gr of morphia had to be given as the patient was again noisy, and delirious. In rational moments he complained of a very severe headache, and backache. Rigidity was marked. It was decided to stop the intrathecal administration of the antimeningococcal serum on account of the poor results obtained.

16/2/19. Patient's condition was much the same except that there was no delirium, and the pain was not so severe.
70 c.cs. of fairly clear Cerebro-Spinal Fluid were withdrawn; but as the antiserum given intrathecally was not

impressing one favourably, and appeared to aggravate the patient's condition, its administration was withheld.
Complement was still absent from the Cerebro-Spinal fluid.

1 = + : 2 = + : 3 = + : 4 = + : 5 = + .

19/2/19. Patient was decidedly improving. Lumbar puncture was again performed, and 50 c.cs. of fairly clear Cerebro-Spinal fluid were withdrawn. On account of the apparent success of not giving any antiserum intrathecally this was again with-held.
Complement had again appeared in the Cerebro- Spinal fluid.

1 = + : 2 = + : 3 = + : 4 = + : 5 = ±

This experiment was twice performed with the same result.
From this date onwards the patient made a rapid, and uninterrupted recovery.

Notes:-1. It is interesting to note that on swabbing the throat of the case prior to his discharge from hospital a type 2 (two) meningococcus was isolated on the 1/4/19. It did not agglutinate at all with type 1 serum in either 1 in 100, 1 in 200, or 1 in 400 dilutions. This agglutination was performed in four separate tests with growths obtained from four separate colonies. In all the results coincided.
It was only with great difficulty that the organism could be kept alive. It was necessary to subculture it on reinforced trypagar three or four times before a sufficient luxuriant growth could be obtained. No growth took place on ordinary trypagar during the first two or three subcultures. This reminds one of the non-success which attended the efforts to subculture the meningococcus when first isolated from the Cerebro-Spinal fluid (vide ante), and also impresses on one the advantage of subculturing for the first two or three times on reinforced trypagar, and not on non- reinforced trypagar as is usually advised. I have found ordinary agar smeared with human blood a most excellent medium on which to cultivate the meningococcus.
For other experimental purposes the organism was kept alive for ten days when it became again necessary to re-agglutinate it. Using the same brands of agglutinating sera it was found that the organism was not only agglutinated by type 2 serum up to 1 in 400 as follows

1 in 100 =++: 1 in 200 =+: 1 in 400 =+.

but was also agglutinated by type 1 serum fully in 1 in 100 dilution, partially in 1 in 200, and not at all in 1 in 400 as follows, 31"

1 in 100 =++: 1 in 200 =+: 1 in 400 = 0.

This agglutination experiment was performed in duplicate with growths obtained from two separate colonies. It seems that the agglutination of a meningococcus may vary after subculture on trypagar.
A similar result has been noticed in connection with the swabbing of carriers at the No: 5 Cerebro- Spinal Contact Centre. One week there may be a meningococcus isolated which gives a pure monovalent agglutination, whilst the next week the meningococcus isolated gives a mixed agglutination, though the agglutinating sera used be the same in both cases.
Of course in the latter case it may be said that, it was a different coccus altogether which was picked off; but from the above experiment it would appear that a change of agglutination characteristics is quite within the bounds of possibility.
How far this may extend it is difficult to say. I have never been able to change in its entirety one type to another either by growing the meningococcus in symbiosis with staphylococci, streptococci or pneumococci, or by growing it on different media at varying temperatures, or by growing it in the presence of various sera, and human secretions e.g. sputum, nasal discharge.

2. This case casts doubts on the advisability of the intrathecal administration of antiserum.
This was always given with great care with regard to speed, and temperature, yet in this case it appeared to aggravate the patient's condition.
The fact that immediate improvement was noted on the cessation of the administration of the antiserum intrathecally may have been a coincidence; but the serious condition of the patient during the intrathecal treatment with the antiserum, and the immediate improvement produced by lumbar puncture, and the withdrawal of the excess of Cerebro-Spinal fluid without any intrathecal medication with antiserum, produced a marked impression on all those

in attendance on the case.
The advisability of early and frequent lumbar puncture with the withdrawal of excess of Cerebro-Spinal fluid is to be noted.

3. Another effect of the intrathecal administration of antiserum was to produce an absolute loss of free complement in the Cerebro-Spinal fluid. This was a marked feature in Case 1.
The cessation of the administration of the antiserum was followed by the return of free complement to the Cerebro-Spinal fluid. This was present on the sixth day after the last intrathecal dose of antiserum; but was not present on the third day after its administration.
Now to consider class 2 viz. cases of late diagnosis, and as it happens of fatal issue. These were three in number.

Case 3. (Pte B) 17/12/18. This man was a repatriated prisoner of War, and arrived in this country with the diagnosis of epilepsy.
He was semi-delirious, and unable to give a history of his illness. This was a typical case of great severity, and probably long duration (emaciation was marked). The chief symptoms were marked double Kernig, head retraction, rigidity of the back, and pain in the back, head and neck. No vomiting was present, and there was also no rash.
Lumbar puncture was performed, and 30c.cs. of turbid Cerebro-Spinal fluid were withdrawn, and 30 c.cs. of pooled 1 and 2 antiserum were given.
Complement was present as follows,

1 = + : 2 = ± : 3 = ± : 4 = ± : 5 = − .

On microscopic examination of the Cerebro-Spinal fluid a gram-negative intracellular diplococcus was found; therefore the case was diagnosed as Cerebro-Spinal Meningitis. On culture, however, no growth could be obtained. The throat swab, blood, and urine were negative for the meningococcus.

19/12/18. Thirty six hours after the first puncture another was done, and 60 c.cs of turbid Cerebro-Spinal fluid were withdrawn, and 30c.cs. pooled 1 and 2 antiserum were given. No growth was obtained from the fluid, and the throat swab was negative.
Complement was absent as follows,

1 = + : 2 = + : 3 = + : 4 = + : 5 = + .

This test was repeated with the same results.

21/12/18. The patient was again lumbar punctured, and 50 c.cs of very turbid Cerebro-Spinal fluid were withdrawn. 30c.cs of pooled 1 and 2 antiserum were given intrathecally. No growth was obtained from the fluid, and no meningococcus was isolated from the throat swab. It is interesting to note that the complement was again present as follows though in diminished amount.

1 = +: 2 = +: 3 = +: 4 = ≠ : 5 = ≠ .

This was tested in three ways viz:-

1. The fluid was kept at atmospheric temperature in the shade.
2. The fluid was kept at 37°C.
3. The fluid was kept in a test tube surrounded by ice in a thermos flask from the time of its withdrawal until the test was performed four hours later.

The results coincided absolutely.

28/12/18. The patient was much worse, and was moribund. 70c.cs of very turbid Cerebro-Spinal fluid were withdrawn. No growth was obtained on any of the culture media used viz. blood agar, reinforced, and non-reinforced trypagar.
35c.cs of pooled types 1 and 2 antiserum were given with 10c.cs of my own fresh serum to neutralise the complement deviating properties of the antiserum. How to find out the proper amounts to use is previously described in the experimental work.
The Cerebro-Spinal fluid withdrawn contained complement as follows,

1 = +: 2 = ≠: 3 = ≠: 4 = ≠ : 5 = ≠ .

The patient's blood serum had a M.H.D. of complement in .25c.c. of a dilution of 1 in 24, and that of my own serum was in a dilution of 1 in 20.

29/12/18. There was no change in the patient's condition. Lumbar puncture was again performed, but no antiserum was administered.
60c.cs of very turbid Cerebro-Spinal fluid were withdrawn.
No complement was present.

1 = + : 2 = + : 3 = + : 4 = + : 5 = + .

30/12/19. Patient died, and post-mortem was refused.

Notes:-1. Failure of the giving of antiserum temporarily deprived of its complement deviating properties is to be noticed.

2. The complement deviating properties of the antiserum is again demonstrated by its effect on the complement content of the Cerebro-Spinal fluid.
That this is a more active case than case 2 is shown by the more rapid return of free complement into the Cerebro-Spinal fluid. Thirty six hours after the first administration no complement was found in the Cerebro-Spinal fluid. Two days after the second administration complement was just beginning to reappear. Seven days after the third administration complement was still increasing in amount, but was not back to the full amount as found on the first withdrawal.
One day after the fourth administration complement had disappeared from the Cerebro-Spinal fluid in spite of the fact that the antiserum, given intrathecally on the previous day, was temporarily deprived of its complement deviating properties by giving it mixed with fresh, human, complement containing serum.

Case 4. Pte.(H)

This case was admitted to hospital, and treated for influenza for about one month before it was diagnosed as Cerebro-Spinal Meningitis. The symptoms had been headache, pain in the back and neck, irregular temperature, and very persistent vomiting.

6/11/18. The complaint of the patient was vomiting, and pain in back, head, and neck.
On examination he was found to be very thin and ill nourished. There was rigidity of the back, and neck, and a double Kernig was present, though not marked.
The patient was lumbar punctured, and given type 2 therapeutic antiserum; the first dose of the serum given being, however, pooled types 1 and 2.
I have unfortunately no notes on the early part of the case as I did not begin my work on the subject until December 1918.
Lumbar puncturing, and the giving of type 2 M.R.C. antimeningococcal serum, however, did not appear to do any good.

20/12/18. The patient was lumbar punctured. Symptoms were as on admission.
60c.cs of what appeared to the naked eye to be clear fluid were withdrawn. On account of this no antiserum was given.

On culture, however, a type two meningococcus was isolated. There was complement present in the Cerebro-Spinal fluid viz,

1 = + : 2 = + : 3 = + : 4 = + : 5 = ±.

24/12/18. Patient was no better except for slight relief of his headache immediately after the last lumbar puncture. I again did lumbar puncture, and slightly opalescent Cerebro-Spinal fluid was withdrawn to the amount of 35c.cs, and 25c.cs of type two antiserum were given intrathecally.
A trace of complement was present in the fluid,

1 = +.: 2 = + : 3 = + : 4 = + : 5 = ±.

A separate test was done by keeping the Cerebro-Spinal fluid in a freezing mixture from the time of its withdrawal until the experiment was performed three and a half hours later.
The result, however, was the same.

28/12/18. The patient had still got the same symptoms though he was running a much higher temperature, and he was becoming very feeble, and emaciated, and his intellect was becoming clouded.
Lumbar puncture yielded 50c.cs. of Cerebro-Spinal fluid under distinct pressure. A type two meningococcus was cultivated from it. The Cerebro-Spinal fluid showed the presence of complement as follows:-

1 = + : 2 = + : 3 = + : 4 = ± : 5 = ±.

It was decided to give the antiserum with excess of complement in it i.e. temporarily devoid of its complement deviating properties.
By previously described experiments it was found that a suitable dose would be to give 25c.cs of type 2 antimeningococcal serum with 10c.cs. of my own fresh serum.
This additional therapeutic measure appeared to have no effect on the patient's condition. During the rest of the patient's illness his condition gradually got worse until his death on 21/3/19.
For the last six weeks of his illness he was mentally unsound.

Notes:-1. This was a typical chronic case.

2. The amount of complement in the Cerebro-

Spinal fluid was very small, probably due to the fact that the lesion was by no means an active one.

3. The complement deviating effect of the antiserum was not shown in this case on account of the long interval between the dose, and the next lumbar puncture- four days.

4. The giving of free complement with the antiserum did not appear to do any more good than by giving the antiserum alone.

5. Wasting was a marked feature of the case. This was probably increased beyond the usual found in Cerebro-Spinal Meningitis by the persistent vomiting, as it was with difficulty that food could be retained in the patient's stomach.

Case 5.
(3rd A/M.S)

15/11/18. This patient was admitted to hospital suffering from what was said to be a relapse after an attack of influenza.

3/12/18. The Doctor-in charge of the case did lumbar puncture, and sent the Cerebro-Spinal fluid to the laboratory. As it was twenty four hours in the post nothing grew on the media; but in the film from the deposit a Gram-negative intracellular diplococcus was found. A diagnosis of Cerebro-Spinal Meningitis was thus arrived at.

4/12/18. The patient was again lumbar punctured, and 40c.cs of turbid Cerebro-Spinal fluid were withdrawn. 20c.cs of pooled types 1 and 2 antiserum were given. The chief symptoms were rigidity of the back, head retraction, double Kernig, severe headache and backache, temperature 104°F, pulse rate about 100 per minute, and there was also occasional vomiting. He showed marked signs of emaciation. A type 2 meningococcus was cultivated. Numerous pus cells, and globulin were present in the Cerebro-Spinal fluid. Complement was present in the Cerebro-spinal fluid as follows:-

1 = + : 2 = ± : 3 = ± : 4 = ± : 5 = ±.

6/12/18. Lumbar puncture yielded 50c.cs. of turbid Cerebro-Spinal fluid. 30c.cs of type 2 antiserum were given. Complement was present as follows:-

1= ± : 2 = ± : 3 = ± : 4 = ± : 5 = ± .

The Doctor in charge of the case began vaccine treatment.

10/12/18. Patient was not doing well.
50c.cs of Cerebro-Spinal fluid were withdrawn, and 30c.cs of type 2 antiserum were given.
Complement was present as follows:-

1 = ± : 2 = ± : 3 = ± : 4 = ± : 5 = ± .

The patient continued i.s.q. The antiserum produced neither ill nor good effects.

31/12/18. I was again called in to see the case. Patient was going downhill. He had numerous blisters present all over the body. No meningococcus could be cultivated from the fluid in these. 30c.cs of Cerebro-Spinal fluid were withdrawn, and 20 c.cs of type 2 antiserum were administered.
Complement was present as follows:-

1 = ± : 2 = ± : 3 = ± : 4 = — : 5 = — .

1/1/19. The patient's condition was much worse after the giving of the last antiserum.

2/1/19. It was decided to give an intravenous injection of antiserum.
A few hours previous to the injection a small dose of antiserum was give subcutaneously. The patient was kept warm in bed both before , during, and after the operation.
The M.H.D. of the complement in the patient's blood serum was 1 in 10.
70c.cs of Cerebro-Spinal fluid were withdrawn by lumbar puncture, and 35c.cs of type 2 antiserum were given intrathecally. 60c.cs of antiserum were also given intravenously with about an equal amount of sterile freshly distilled .8% saline solution. During the administration of the antiserum intravenously , in spite of every care being taken as to speed, pressure, and temperature of fluid given, the patient got a little cyanosed, and the pulse rate increased in frequency. This, however, was only transient. About half to three quarters of an hour after the administration the patient began to suffer from anaphylactic shock. The pulse rate increased, and the pulse became very feeble, being imperceptible at the wrist. Temperature rose to 102°F. and cyanosis was most marked.
The Doctor in charge of the case gave the patient Oxygen inhalations, and

strychnine hypodermically, and the patient gradually recovered.
For the next ten days the Doctor said there was a marked improvement in the case, and he began to hope for a favourable issue; but after that time the patient returned to his former state. Considering the severity of the shock I did not feel justified in recommending another intravenous injection of the antiserum.
Complement in this case was present in the Cerebro-Spinal fluid as follows:-

1 = ± ; 2 = ± ; 3 = ± ; 4 = ± ; 5 = ± .

21/1/19. The patient was now very thin, and emaciated, and slowly sinking.
Lumbar puncture produced 30c.cs of Cerebro-Spinal fluid, and 20c.cs of antiserum (type2) were given.
Complement was present as follows:-

1 = + ; 2 = ± ; 3 = ± ; 4 = ± ; 5 = ± .

22/1/19. As no improvement occurred another 35c.cs of turbid Cerebro-Spinal fluid were withdrawn, and 20c.cs of type 2 antiserum were given.
Complement was present in the Cerebro-Spinal fluid as follows:-

1 = + ; 2 = + ; 3 = + ; 4 = ± ; 5 = ± .

No improvement occurred, and patient died on 28/1/19.

Notes:- It is to be noticed that in the last two lumbar punctures a relatively small amount of Cerebro-Spinal fluid was withdrawn, and not even temporary relief of the pressure symptoms was obtained. This is accounted for by the fact that at the post-mortem a certain amount of hydrocephalus was found.
The lesion in this case was very active, and the patient especially in the early days of his illness showed signs, and symptoms of an acute illness. This is shown by considering the effect of the antiserum on the complement content of the Cerebro-Spinal fluid.

1. Though given 20c.cs of pooled types 1 and 2 antiserum on the 4/12/18 yet on the 6/12/18 (two days later) the Cerebro-Spinal fluid contained as much complement as it did before the antiserum was administered.

2. This can be said about the result of -

giving 30c.cs of type 2 antiserum on the 6/12/18, and testing the Cerebro-Spinal fluid for complement on the 10/12/18 i.e. four days later.

3. By not giving any antiserum between the 10/12/18 and 31/12/18 i.e. for twenty days, the complement content of the Cerebro-Spinal fluid had risen i.e. the amount of complement in a definite volume of Cerebro-Spinal fluid. It is impossible to calculate the total amount as, for the purpose, one would require to know accurately the total amount of Cerebro-Spinal fluid in the Cerebro-Spinal system at each time the fluid is tested.

4. On the 21/1/19 20c.cs. of antiserum (type 2) were given, and on the following day the Cerebro-Spinal fluid showed a diminution of complement as shown by comparing the results of the test for complement present in the Cerebro-Spinal fluid withdrawn on the 21/1/19 with those obtained by testing the Cerebro-Spinal fluid withdrawn on the 22/1/19.

The last (4) again demonstrates the complement deviating properties of the antiserum.

Another point about the case was the effect on the patient of an intravenous injection of the antiserum. Therapeutically it impressed one i.e. if the anaphylactic shock be disregarded, as a worthy adjunct in the treatment even in a desperate case such as this.

Post-Mortem. was allowed, and the following was found on examining the brain and spinal cord.

The membranes of the brain, and spinal cord were thickened with inflammatory changes. This was especially marked in the lumbar region of the cord, and the basal region of the brain. A more modified inflammatory change was, however, also present over the vertex of the brain. The ventricular system was distended, and showed a commencing hydrocephalus. It was calculated that about 130c.cs of fluid were present in the Cerebro-Spinal system. The fluid was turbid.

The brain lining the ventricles was soft, and it was found that both the Foramen of Majendie, and the lower end of the Aqueductus cerebri (Sylvius) were blocked thus producing the above mentioned condition of hydrocephalus. This accounts for the lack of even temporary relief after the last two lumbar punctures.

Special attention was paid to microscopic sections of the brain lining the ventricles. In places the ependyma had disappeared, and the surface layer of the brain tissue showed marked oedema with degenerative changes in both nerve and neuroglia cells. The blood vessels were markedly distended, and there was a very obvious peri-vascular infiltration with round cells.
This change gradually became less marked as it was traced into the substance of the brain tissue. The membranes of the cord, and brain showed a more diffuse infiltration with round cells, though here again perivascular infiltration was obvious.
The vessels especially of the pia mater were greatly distended.
The inflammatory changes were much more marked in the pia-archnoid than in the dura mater.

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SUMMARY.

1. Complement is present in the Cerebro-Spinal fluid in untreated cases of Cerebro-Spinal Meningitis.

2. The amount appears to vary with the activity and extent of the meningeal lesion.

3. The M.R.C. antimeningococcal serum, when given intrathecally, removes or diminishes complement, for a variable time, from the Cerebro-Spinal fluid of cases of Cerebro-Spinal Meningitis.

4. This removal of complement is produced by complement deviation, and not by complement fixation.

5. The giving of antiserum, which is temporarily deprived of its complement deviating properties (i.e. by the addition of complement containing, fresh human serum), is not a success.

6. The possible reason of this is that though the antiserum be saturated with complement, yet on the disappearance of free complement there is a partial return of the complement deviating properties.

7. Filtration of the antiserum through a Berkefeld filter and

Doulton filter removes some of the complement deviating properties.

8. A new line of treatment is indicated-. the rough outline of this being as follows:- "41"

The antiserum is to be given intravenously, and the effect kept up by giving it either subcutaneously, intramuscularly ,or intravenously. The Cerebro-Spinal system is to be drained by frequent lumbar puncture, and if necessary the flow of serum into the Cerebro-Spinal system is to be increased by use of intrathecal doses of hypertonic saline.

9. The giving of 10% sterile hypertonic saline solution intrathecally in 4.0 c.cs quantities does not appear to have any deleterious effect on rabbits.

10. In giving intravenous injections of antisera to rabbits the following well known points are emphasied- the fluid is to be given at the body temperature; it is to be given slowly, and at a low pressure, and the animal should be kept warm both before, during, and after the administration.

11. In considering the treatment of the cases the want of success attending the administration of the M.R.C's antimeningococcal serum intrathecally is indicated. The success of drainage of the Cerebro-Spinal system is on the other hand noticed.

12. A few remarks are made on the possibility of the mutation of the agglutination characteristics of the meningococcus.

Before closing this paper I wish to tender my sincere thanks to Dr. C.Fletcher for his kindness in reading over my work, and giving me the benefit of his excellent criticism.

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J.W.E.