vomiting, until his admission into the Manchester Royal Infirmary on the fourth day. When admitted his temperature was 101.2°F . and his pulse was 84. The abdomen was somewhat distended, with general tenderness and resistance, most marked on the right side. Above the outer part of Poupart's ligament a distinct induration could be felt. In the course of three or four days the general distension and tenderness subsided under the application of belladonna and fomentations, and the temperature became normal. The induration above Poupart's ligament also gradually disappeared but a distinct thickening remained in the situation of the appendix and could still be detected at the time of his discharge three weeks subsequently, when he was sent to the convalescent home in the country. He came to show himself about a month later and the region of the appendix was then quite free from any swelling. Up to the present time, four months subsequently, there has been no further trouble.

It is also probable that without the application of any direct violence traumatism may excite an attack of appendicitis in another manner—e.g., a severe strain, by causing a sudden and forcible contraction of the abdominal muscles, may separate or break down adhesion between the appendix and the abdominal wall, causing an extravasation of blood either into or around the appendix, or possibly producing a rupture of its wall and allowing its contents to escape. Kelly and Hurdon state that in ten cases of appendicitis following an injury, where no concretion was found, there were evidences of old adhesions in seven. The following case probably belongs to this class, it being an established fact that mild attacks of appendicitis are often followed by the formation of firm adhesions between the appendix and adjacent structures. Statistics also show that adhesions are frequently found at necropsies where there has been no history of any attacks of appendicitis occurring during life.

CASE 4.—A boy, aged 15 years, who in the preceding nine months had suffered from two mild attacks of abdominal pain and also from occasional slight "bilious" attacks, whilst out fishing strained himself in jumping across a stream and was at once seized with severe pain in the abdomen. The pain continued and in the evening he complained of feeling unwell. Symptoms of acute appendicitis developed and when seen in consultation later in the evening of the third day he presented typical symptoms of general peritonitis. The temperature was 101°F . and the pulse was 130. There was general distension of the abdomen with dulness in each flank; over the region of the appendix there was slight increase of dulness with more resistance than on the opposite side. On the following morning the abdomen was opened and was found to be full of pus. The boy's condition was too serious to allow of any search being made for the appendix and no concretion was seen. Death took place about 12 hours after the operation. No necropsy was made.

Manchester.

ON THE MULTIPLICITY OF COMPLEMENTS IN BACTERIOLYTIC SERA.

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OF recent years most of the leaders of science have turned to hæmolysis in vitro for the purpose of ascertaining the general truths underlying lysogenic action. And whilst it will be readily admitted that this indirect method at present offers the best possibilities of obtaining firm foothold for an attack on the problem of bacteriolysis, yet none the less the ultimate solution of this latter problem will only be obtained by testing directly those general principles that may have been obtained by indirect methods. Further, for those who are not prepared to admit that the laws of hæmolysis and bacteriolysis are identical until they have been proved to be so, any direct attempt to solve the problem of bacteriolysis has this great advantage that results so obtained can only be invalidated on the grounds of faulty technique, erroneous

observations, conceptions, or conclusions. Results so obtained cannot, however, be discredited on the ground of their incompatibility with results obtained by indirect methods such as hæmolysis.

The literature on the subject of immunity has now attained to such vast proportions that any attempt at a critical *résumé* would be quite out of place in a communication of this nature. For the purposes of this paper, however, a very brief sketch will suffice. The present state of our knowledge with regard to bacteriolysis was summarised as follows in the presidential address¹ to the Section of Pathology at the Oxford meeting of the British Medical Association in 1904:

We must recognise that it is limited to the statement that the killing of the bacteria is associated with the presence in the serum of an immune animal of two substances, one thermolabile (complement) and the other thermostable (immune body). There is this further fact that we are only certain of the specificity of the immune body—that is, we know that immune body will only act deleteriously on the protoplasm which stimulated its genesis. We cannot make a similar statement regarding complement.

If this statement be enlarged so as to include normal sera it will represent fairly accurately the state of our knowledge at the present day. With regard to the complement, it may be briefly stated that as the result of different conceptions as to the nature of lysogenic action there are two great schools of thought, one of which holds that there are at most not more than two complements, one being concerned in the lysis of bacteria and the other in the lysis of cells generally. The other school maintains that there are many different complements differing in their relations to the various amboceptors. The supporters of the pluralistic doctrine, working on the principle that identical chemical substances should give identical results with the same reagent, have employed the methods of differential heating, differential filtration, selective absorption, the use of anti-complements—natural and artificial—treatment with neutral substances, such as silica, digestion with ferments as papain, and treatment with alkalis, &c., to prove their contention. The result of their labours has been to give a commanding position to the doctrine of the multiplicity of complements in hæmolytic sera. With regard to bacteriolytic sera, it was shown by Wright and Windsor,² that if to a serum normally bactericidal for the bacillus typhosus a sterilised typhoid or cholera culture be added, the antibactericidal effect which is in each case obtained is obtained indifferently with either variety of sterilised culture. These observers argued from this that either the bactericidal substance in the serum which kills the typhoid bacillus is one and the same substance which kills the cholera vibrio, or alternatively that the bactericidal substance which kills the cholera vibrio possesses an element in common with the bactericidal substance which kills the typhoid bacillus. As the result of further experiments they concluded "that the bactericidal effects of a serum, at any rate in the case of a serum derived from the immunised animal, is achieved by the coöperation of two bactericidal elements, one of these being a chemical agent which exerts an action on more than one species of micro-organism, and the other a chemical agent which is specific for each particular species of micro-organism." Longcope,³ working in the Ayer Clinical Laboratory, Pennsylvania Hospital, came to a very different conclusion. The method of this observer was to estimate the amount of unheated human serum necessary to reactivate one cubic centimetre of heated human serum for bacillus typhosus and bacillus coli respectively, standard quantities of emulsified agar cultures of these two organisms being used for the test. In the case of normal persons it was found that one-twentieth of a cubic centimetre of unheated serum was sufficient to re-activate one cubic centimetre of heated serum for a standard quantity of a typhoid emulsion. For the same quantity of the coli emulsion six-tenths of a cubic centimetre of unheated serum were necessary to re-activate one cubic centimetre of heated serum. In the case of the blood of persons suffering from typhoid fever it was found that the re-activating power of the serum was greatly diminished for typhoid bacilli and not for colon bacilli. The re-activating power of the sera of these typhoid patients was also tried against bacillus paracoli and bacillus dysenteriae, and it was found that the sera showed a decreased re-activating power for typhoid bacilli alone. From this Longcope inferred the existence of a specific typhoid complement and concludes from his work that human blood

¹ Brit. Med. Jour., Sept. 7th, 1904.

² Journal of Hygiene, October, 1902.

³ Ibid., January, 1903.

serum contains a multiplicity of bacteriolytic complements. Longcope's observations are, however, very meagre, his conclusions being based on the examination of the blood of only three typhoid patients. Of these three cases the result of the examination of the blood of one is hardly in accordance with his conclusions, as in the tables it is shown that one-twentieth of a cubic centimetre of unheated serum was able to re-activate one cubic centimetre of heated serum, this being the standard laid down by him for normal serum. Further, he altogether neglected to consider the phenomenon of "Komplimentablendung." The object of the present investigation was to ascertain definitely the existence or non-existence of a specific typhoid complement in the serum of an immunised animal, the serum being normally bactericidal for both bacillus typhosus and the cholera vibrio.

Collection and storage of serum.—The animal selected for the supply of serum was the goat which in India is one of the most useful of laboratory animals. A large external jugular vein covered by a thin integument permits of the easy withdrawal of samples of blood under aseptic conditions. Further, relatively large quantities of blood can be withdrawn at short intervals without inconveniencing the animal and with no more discomfort than that occasioned by the prick of a hypodermic needle. The procedure adopted was as follows: The neck of the animal having been shaved over the region of the external jugular vein and scrubbed with 5 per cent. carbolic acid the needle of a sterilised hypodermic syringe of 20 cubic centimetres capacity was introduced into the jugular vein and the syringe was filled with blood. The syringe was then withdrawn and the needle was unshipped from the barrel of the syringe with sterilised forceps. The contents of the syringe were then transferred to a sterile conical glass vessel of about 50 cubic centimetres capacity and provided with a metal cover which fitted after the manner of the upper lid of a Petri dish. After the blood had clotted the clot was loosened by passing a thin glass rod between the clot and the sides of the vessel. The vessel was then placed aside for 24 hours, after which the serum was ready for experiment. I have kept serum collected and stored in this way for months without bacterial development taking place. The same goat was used throughout this investigation except where otherwise stated.

Technique for the estimation of the bactericidal power of the serum.—The technique employed was based on that introduced by my former teacher, Dr. A. E. Wright,⁴ in connexion with his researches on the bactericidal power of human serum. The stem of the diluting pipette was graduated in ten divisions of five cubic millimetres each, the remaining graduation marks indicating 450 and 500 cubic millimetres respectively. I have on a previous occasion⁵ alluded to the general utility and accuracy of this modification of Wright's original pipette. When quantities such as 200 and 250 cubic millimetres were being used small pipettes graduated for these quantities by means of Wright's method were used. Dilutions of the test broth cultures employed were made in sterile watch-glasses as advised by Wright.

TABLE I.—*Giving the Quantities found most convenient for making the Dilutions employed.*

Dilution.	Culture.	Broth.
2 fold.	100 cubic millimetres.	100 cubic millimetres.
5 "	50 " "	200 " "
10 "	50 " "	450 " "
25 "	10 " "	240 " "
50 "	10 " "	490 " "
100 "	5 " "	495 " "
1,000 "	50 cubic millimetres of 100-fold dilution.	450 " "
10,000 "	50 cubic millimetres of 1000-fold dilution.	450 " "
100,000 "	50 cubic millimetres of 10,000-fold dilution.	450 " "
1,000,000 "	50 cubic millimetres of 100,000-fold dilution.	450 " "
100,000,000 "	10 cubic millimetres of 1,000,000-fold dilution.	990 " "

Culture enumeration was made when necessary from the 100,000,000-fold dilution in the way described by Wright.⁶ In order to test the bactericidal power of the test serum employed equal quantities of the undiluted test serum and an ascending series of dilutions of a 24 hours broth culture of the test organism were brought together in small test-tubes. These test-tubes were made from glass tubing, being blown and lipped in the ordinary way. The average dimensions of these tubes were as follows: length, 4 5 to 5 centimetres; capacity about 1 cubic centimetre. When using larger quantities of serum than 50 cubic millimetres the bottoms of these tubes were given a bulbous shape, the extreme diameter of the bulb being about a centimetre. The steps of the technique were as follows. Tubes were arranged in racks according to requirements and then labeled with a grease pencil. Measured quantities of the test serum were then filled into the tubes of each series. Appropriate dilutions of a 24 hours broth culture of the test organism were then made in a series of sterile watch glasses and the culture enumerated when necessary. Equivalent portions of each dilution, beginning at the highest dilution, were then added to the tubes containing the test serum. During this part of the technique care must be taken not to soil the sides of the tubes when adding the dilutions of the test organism. If this precaution be not observed irregular results will be obtained. It is for this reason that the tubes are given a bulbous shape, as by this device the capacity of the tubes is increased without increasing the length. After the dilutions of the test organism have been added to the series of tubes containing the test serum the contents of the tubes were thoroughly mixed by shaking. The tubes were then incubated at 37° C. for from 18 to 24 hours. At the end of this time to each tube 0.5 cubic centimetre of sterile broth was added with the pipette, beginning at that tube which contained the highest dilution of the test organism. The tubes were then incubated at 37° C. for 48 hours, after which the bactericidal power of the test serum for the test organism was ascertained by observing the last tube in the series which showed growth. In the tables in this paper the term "growth" means that the growth obtained presented the characters of a pure culture of the test organism. In cases of doubt the purity of the culture was ascertained by the usual methods. The same strains of bacillus typhosus and spirillum cholerae Asiaticæ were used throughout these experiments. The strain of bacillus typhosus employed was isolated locally from a case of typhoid fever, whilst the strain of spirillum cholerae Asiaticæ was supplied from the Parel Laboratory, Bombay. This latter strain was isolated from an acute case of cholera in Baroda in 1900. Sterility controls for serum, &c., were always put up and only those experiments are recorded in which these controls proved sterile.

The first step in the investigation was to ascertain the bactericidal power of normal goat's serum 24 hours old in contact with clot for bacillus typhosus and spirillum cholerae Asiaticæ. The result is shown in Table II. In addition I may state that for most examples of the bactericidal power of normal human serum for cholera and typhoid fever published by Wright in his various papers I can produce a parallel example from the series of goats tested by me from time to time during the past year. Normal goat's serum 24 hours old in contact with clot is comparable with normal human serum so far as its bactericidal power for typhoid fever and cholera is concerned.

We have next to consider the changes which occur in the serum when kept in contact with clot. When stored in contact with the clot it was found that if the serum were examined 24 hours after the blood had been withdrawn and again after 48 hours the bactericidal power of the serum had undergone little or no change. In some cases there was apparently a slight increase of bactericidal power (Table II.).

The application of the principle of selective absorption to the solution of the problem.—In applying the principle of selective absorption it will first be necessary to ascertain the relative quantities of a given amboceptor and its complement in a given serum unit. If in the serum unit it should be found that the quantity of the given amboceptor present is such that it is unable to insure that all the complement capable of combining with it shall be used up it will be necessary to artificially increase the amount of amboceptor to the necessary strength. Having selected the typhoid

⁴ Proceedings of the Royal Society, 1902; THE LANCET, July 1st, 1901, p. 1502.

⁵ THE LANCET, June 17th, 1905, p. 1641.

⁶ THE LANCET, June 1st, 1901, p. 1532.

TABLE II.—Showing the Bactericidal Power of Normal Goat's Serum 24 hours old for (1) *Bacillus Typhosus* and for (2) *Spirillum Cholerae Asiaticæ*.

Dilutions in which the 24 hours old living broth cultures were employed.	50 c.mm. N.G.S.* 24 hours old and 50 c.mm. of a dilution of a typhoid culture.	50 c.mm. N.G.S. 24 hours old and 50 c.mm. of a dilution of a cholera culture.	50 c.mm. N.G.S. 24 hours old and 50 c.mm. of a dilution of a typhoid culture containing 165,000,000 organisms per c.c.	50 c.mm. N.G.S. 48 hours old and 50 c.mm. of a dilution of a typhoid culture containing 955,000,000 organisms per c.c.
Undiluted culture ...	Growth	Growth	Growth	Growth
2 fold dilution	"	Sterile	"	"
5 " "	"	"	Sterile	Sterile
10 " "	"	"	"	"
25 " "	"	"	"	"
50 " "	"	"	"	"
100 " "	Sterile	"	"	"
1,000 " "	"	"	"	"
10,000 " "	"	"	"	"
100,000 " "	"	"	"	"
1,000,000 " "	"	"	"	"

* Normal goat's serum.

amboceptor and 50 cubic millimetres of normal goat's serum 24 hours old in contact with clot for the amboceptor and serum unit respectively, experiments to this end were conducted as follows. 30 small sterile test-tubes arranged in series of ten were each charged with 50 cubic millimetres of normal goat's serum 24 hours old. To each tube of Series 2 50 cubic millimetres of the same serum which had previously been heated to 55° C. for half an hour were added, thereby doubling the amount of typhoid amboceptor originally present in the 50 cubic millimetres of unheated serum. To each tube of Series 3 100 cubic millimetres of heated normal goat's serum were added, thereby trebling the amount of the typhoid amboceptor. Ascending dilutions of a 24 hours broth culture of bacillus typhosus were added to each series as set forth in Table III. The net result of these experiments was to

TABLE III.—Showing the Results of Addition of *Bacillus Typhosus* Culture.

Dilutions of the 24 hours old living broth culture of bacillus typhosus employed as a test containing 430,000,000 organisms per c.c.	50 c.mm. N.G.S.* 24 hours old and 50 c.mm. of a dilution of the typhoid culture.	50 c.mm. N.G.S. 24 hours old, 50 c.mm. H.N.G.S.† and 50 c.mm. of a dilution of the typhoid culture.	50 c.mm. N.G.S. 24 hours old, 100 c.mm. H.N.G.S., and 50 c.mm. of a dilution of the typhoid culture.
Undiluted culture ...	Growth	Growth	Growth
2 fold dilution ...	"	"	"
5 " "	"	"	"
10 " "	"	Sterile	Sterile
25 " "	"	"	"
50 " "	"	"	"
100 " "	"	"	"
1,000 " "	Sterile	"	"
10,000 " "	"	"	"
100,000 " "	"	"	"
1,000,000 " "	"	"	"

* Normal goat's serum.

† Heated normal goat's serum.

show that doubling the amboceptor produced the limit increase of bactericidal power but that trebling the amboceptor did not, as a rule, decrease the bactericidal power. A typical experiment is recorded in Table II. In view of these results it was decided to treble the amount of typhoid amboceptor in the following experiments in order to insure

the combination of all complements in the serum unit capable of combining with it.

We have next to consider the procedure necessary for the removal from the serum unit of all the complement capable of combining with the typhoid amboceptor. In conducting experiments to this end the artificially prepared serum unit (two parts heated serum, one part unheated serum) was saturated with a sterilised emulsion of a 24 hours agar culture of bacillus typhosus and placed in the incubator for varying periods of time. The mixture was then centrifugalised and the bactericidal power of the supernatant fluid for typhoid fever and cholera ascertained. The details of the technique were as follows. A sterile centrifuge tube provided with a sterile rubber cap was prepared containing one cubic centimetre of normal goat's serum, 24 hours old; two cubic centimetres of the same serum heated (H.N.G.S.); and one cubic centimetre of a sterilised emulsion of bacillus typhosus. The sterilised emulsion of bacillus typhosus was prepared by emulsifying the whole of the 24 hours agar slope culture with two cubic centimetres of sterile normal salt and then heating to from 60° to 64° C. for 15 minutes. As I have never been able to obtain from my goat by natural or artificial means a sample of serum capable of sterilising an equal volume of a 24 hours broth culture of bacillus typhosus the quantity of the emulsion added to the centrifuge tube was considered sufficient to insure the removal therefrom of all the complement capable of combining with the typhoid amboceptor. For a control a sterile test-tube was prepared containing one cubic centimetre of normal goat's serum 24 hours old, two cubic centimetres of heated normal goat's serum; and one cubic centimetre of sterile normal salt. The centrifuge tubes were then incubated at 37° C. for different periods of

TABLE IV.—Showing the Bactericidal Power of Supernatant Fluid.

After 15 minutes' incubation.

Dilutions of the 24 hours old living broth cultures employed.	200 c.mm. of Sn.F.* and 50 c.mm. of a dilution of a typhoid culture.	200 c.mm. of C.F.† and 50 c.mm. of a dilution of a typhoid culture.	200 c.mm. of Sn.F. and 50 c.mm. of a dilution of a cholera culture.	200 c.mm. of C.F. and 50 c.mm. of a dilution of a cholera culture.	200 c.mm. of sterile broth and 50 c.mm. of a dilution of a cholera culture.
100 fold dilution	Growth	Sterile	Sterile	Sterile	Growth
1,000 " "	"	"	"	"	"
10,000 " "	"	"	"	"	"
100,000 " "	"	"	"	"	"
1,000,000 " "	"	"	"	"	"

After 25 minutes' incubation.

100 fold dilution	Growth	Growth	Sterile	Sterile	Growth
1,000 " "	"	Sterile	"	"	"
10,000 " "	"	"	"	"	"
100,000 " "	"	"	"	"	"
1,000,000 " "	"	"	"	"	"

After 30 minutes' incubation.

100 fold dilution	Growth	Growth	Sterile	Sterile	Growth
1,000 " "	"	"	"	"	"
10,000 " "	"	Sterile	"	"	"
100,000 " "	"	"	"	"	"
1,000,000 " "	"	"	"	"	"

After one hour's incubation.

100 fold dilution	Growth	Sterile	Growth	Sterile	Growth
1,000 " "	"	"	"	"	"
10,000 " "	"	"	"	"	"
100,000 " "	"	"	"	"	"
1,000,000 " "	"	"	"	"	"

After two hours' incubation.

100 fold dilution	Growth	Sterile	Growth	Sterile	Growth
1,000 " "	"	"	"	"	"
10,000 " "	"	"	"	"	"
100,000 " "	"	"	"	"	"
1,000,000 " "	"	"	"	"	"

* Supernatant fluid.

† Control fluid.

time, varying from 15 minutes to two hours. After incubation the centrifuge tube was centrifugalised and the supernatant fluid pipetted off. The supernatant fluid (Sn.F.) and the control fluid (C.F.) were then tested for bactericidal power against ascending dilutions of a typhoid culture and a cholera culture, as set forth in Table IV. As both the supernatant fluid and the control fluid represent a fourfold dilution of the original serum unit 200 cubic millimetres of each were used to represent the serum unit of 50 cubic millimetres. Sterility controls for supernatant fluid and control fluid were always put up and only those experiments are recorded in which these controls proved sterile. A reference to the tables at once shows the importance of the time factor in experiments of this nature. Two hours' incubation yields a supernatant fluid devoid of bactericidal power for both typhoid fever and cholera. Incubation for one hour gives a similar result. Incubation for half an hour, 25 minutes, and 15 minutes yields, however, a supernatant fluid which, whilst devoid of bactericidal power for typhoid fever, is still strongly bactericidal for cholera. These experiments contain several obvious fallacies. In the first place, the loss of bactericidal power for typhoid fever resulting from 15, 25, and 30 minutes incubation may be due to the abstraction of the specific amboceptor, complement having been left behind; and secondly, these experiments do not enable us to state whether or not incubation for the corresponding periods has in any way diminished the bactericidal power of the supernatant fluid for cholera as compared with that of the control fluid. A second series of experiments was conducted for the purpose of settling these points. An extra series of tubes of the supernatant fluid, fortified by the addition of 50 cubic millimetres of heated normal goat's serum to each tube to replace amboceptor, was put up when testing the bactericidal power of the supernatant fluid for typhoid fever. At the same time a complete estimation of the bactericidal power for cholera of both the supernatant fluid and the control fluid was made (Table V.). The results are very striking. It will be seen

TABLE V.—Showing the Bactericidal Power of Supernatant Fluid after 15 Minutes' Incubation.

Dilution of the 24 hours old living broth cultures employed.	200 c.mm. Sn.F.* and 50 c.mm. of a dilution of a typhoid culture.	200 c.mm. Sn.F., 50 c.mm. H.N.G.S.† and 50 c.mm. of a dilution of a typhoid culture.	200 c.mm. C.F.‡ and 50 c.mm. dilution of a typhoid culture.	200 c.mm. Sn.F. and 50 c.mm. of a dilution of a cholera culture.	200 c.mm. C.F. and 50 c.mm. of a dilution of a cholera culture.	200 c.mm. sterile broth and 50 c.mm. of a dilution of cholera culture.
Undiluted culture...	—	—	—	Growth	Growth	—
2 fold dilution	—	—	—	"	"	—
5 " "	—	—	—	"	"	—
10 " "	—	—	—	"	"	—
25 " "	—	—	—	"	Sterile	—
50 " "	—	—	—	"	"	—
100 " "	Growth	Growth	Growth	"	"	Growth
1,000 " "	"	"	"	"	"	"
10,000 " "	"	"	Sterile	Sterile	"	"
100,000 " "	"	"	"	"	"	"
1,000,000 " "	"	"	"	"	"	"

* Supernatant fluid. † Heated normal goats' serum. ‡ Control fluid.

that after 15 minutes' incubation the addition of heated serum to the supernatant fluid does not restore its bactericidal power for typhoid fever, whilst the bactericidal power of the supernatant fluid for cholera has undergone considerable diminution as compared with that of the control fluid. Viewed broadly, these experiments warrant the statement that after 15 minutes' incubation of a mixture of serum and sterilised typhoid emulsion prepared in the way previously described the bactericidal power of the supernatant fluid is completely removed for typhoid fever, whilst it is impaired for cholera, the phenomenon not being due to abstraction of amboceptor only. If the mixture be incubated for one hour or longer then all the bactericidal power for both typhoid fever and cholera has been completely removed. These

experiments admit of at least two explanations. One is that we have here evidence of the existence of two complements analogous to the dominant and non-dominant complements of Ehrlich in hæmolytic sera, with the proviso that the combination between amboceptor and dominant complement is a necessary antecedent to the combination of amboceptor and non-dominant complement. The other is that we are dealing with only one complement. In this case the experiments could be explained by stating that after 15 minutes' incubation so much complement had been absorbed that the remainder was in such dilution that the combination of bacillus typhosus + typhoid amboceptor + complement could not take place unless bacillus typhosus was present in very large quantities. The dilution of the remaining complement was not, however, sufficient to prevent the bactericidal action for cholera, which presumably can make use of complement in a much higher dilution than bacillus typhosus. As we might expect after the lapse of 15 minutes' incubation, sufficient complement is removed by the typhoid amboceptor to reduce considerably the bactericidal action for cholera. If the mixture be incubated for longer periods of time increasing quantities of complement are fixed by the typhoid amboceptor until at length all the complement has been fixed and the bactericidal action of the supernatant fluid for both cholera and typhoid fever completely removed in consequence. It becomes then a point of importance to determine which of these two explanations is the correct one. In this connexion experiments were conducted for the purpose of ascertaining what occurs if after 15 minutes' incubation the mixture be centrifugalised, the supernatant fluid at once re-saturated with a sterilised emulsion of bacillus typhosus, and re-incubated for 45 minutes. If the theory of a dominant and non-dominant complement be the correct one then re-saturation and re-incubation should not materially reduce the bactericidal power of the supernatant fluid for cholera as compared with that of the supernatant fluid after 15 minutes' incubation. If, however, the single complement theory be the correct one then we should expect that the bactericidal power of the supernatant fluid against cholera after re-saturation and re-incubation would be steadily reduced and ultimately completely removed. The steps of the procedure were as follows. Two sterile rubber-capped centrifuge tubes were prepared, each containing one cubic centimetre of normal goat's serum 24 hours old; two cubic centimetres of heated normal goat's serum; and one cubic centimetre of sterilised typhoid emulsion. For controls two sterile test-tubes were prepared, each containing one cubic centimetre of normal goat's serum 24 hours old; two cubic centimetres of heated normal goat's serum; and one cubic centimetre of normal salt. These tubes were labeled "C.F. 1" and "C.F. 2." The two centrifuge tubes and the two test tubes were placed in the incubator at 37° C. After 15 minutes' incubation all four tubes were removed and the centrifuge tubes were at once centrifugalised. The supernatant fluid was then pipetted off into two sterile test-tubes. The supernatant fluid in one of these tubes was labeled "Sn.F. 1" and placed on one side, together with the control tube labeled "C.F. 1." The remaining supernatant fluid was then re-saturated with one cubic centimetre of sterilised typhoid emulsion and filled into a sterile rubber-capped centrifuge tube. One cubic centimetre of sterile normal salt was then added to the control tube labeled "C.F. 2" and both the centrifuge tube and the control tube were returned to the incubator. The bactericidal action of "Sn.F. 1" and "C.F. 1" was then tested against cholera and typhoid fever. After 45 minutes' re-incubation the centrifuge tube and the control tube were removed from the incubator. The centrifuge tube was at once centrifugalised and the supernatant fluid was pipetted off and denoted as "Sn.F. 2." The bactericidal power of "Sn.F. 2" and "C.F. 2" was then tested for both typhoid fever and cholera. The result is set forth in Table VI. It will be seen that after 45 minutes' re-incubation the bactericidal power of Sn.F. 2 for cholera has been practically completely removed. This militates against the theory of a dominant and non-dominant complement. The fact that 45 minutes' re-incubation still leaves Sn.F. 2 with a trace of bactericidal action for cholera is easily explicable on the ground that the absorptive action is naturally a little slower after the process has been stopped and has to be re-started. These experiments, I think, suggest a reconsideration of the theory of a specific typhoid complement and indicate that the complement for typhoid fever and cholera is probably one and the same.

TABLE VI.—Showing the Bactericidal Power of Supernatant Fluids Nos. 1 and 2.

No. 1, after 15 minutes' incubation.						
Dilutions of 24 hours old living broth cultures employed.	200 c.mm. Sn.F. 1* and 50 c.mm. of a dilution of a typhoid culture.	200 c.mm. Sn.F. 1, 50 c.mm. H.N.G.S.,† and 50 c.mm. of a dilution of a typhoid culture.	200 c.mm. C.F. 1† and 50 c.mm. dilution of a typhoid culture.	200 c.mm. Sn.F. 1 and 50 c.mm. of a dilution of a cholera culture.	200 c.mm. C.F. 1 and 50 c.mm. of a dilution of a cholera culture.	200 c.mm. sterile broth and 50 c.mm. of a dilution of a cholera culture.
10 fold	—	—	—	Growth	Sterile	—
25 "	—	—	—	"	"	—
50 "	—	—	—	"	"	—
100 "	Growth	Growth	Growth	Sterile	"	Growth
1,000 "	"	"	"	"	"	"
10,000 "	"	"	Sterile	"	"	"
100,000 "	"	"	"	"	"	"
1,000,000 "	"	"	"	"	"	Sterile

* Supernatant fluid No. 1. † Heated normal goat's serum.
‡ Control fluid No. 1.

No. 2, after 45 minutes' re-incubation.					
Dilutions of the 24 hours old living broth cultures employed.	250 c.mm. Sn.F. 2* and 50 c.mm. dilution of a typhoid culture.	250 c.mm. C.F. 2† and 50 c.mm. of a dilution of a typhoid culture.	250 c.mm. Sn.F. 2 and 50 c.mm. of a dilution of a cholera culture.	250 c.mm. C.F. 2 and 50 c.mm. of a dilution of a cholera culture.	250 c.mm. sterile broth and 50 c.mm. of a dilution of a cholera culture.
10 fold dilution	—	—	Growth	Sterile	—
25 "	—	—	"	"	—
50 "	—	—	"	"	—
100 "	Growth	Growth	"	"	Growth
1,000 "	"	"	"	"	"
10,000 "	"	Sterile	"	"	"
100,000 "	"	"	Sterile	"	"
1,000,000 "	"	"	"	"	Sterile

* Supernatant fluid No. 2. † Control fluid No. 2.

The application of the principle of "Komplementablenkung" to a solution of the problem.—Opinions differ widely as to the real interpretation of this phenomenon, but as a *résumé* of current opinions is now to be found in modern text-books⁷ it will be unnecessary to go deeply into the matter here. Suffice it to say that of late it has come to be regarded as a phenomenon depending in some way or other on an interference with the normal action of the complement when an excess of amboceptor is added to a bacteriolytic serum. The phenomenon is not confined to bacteriolysis as it has also been observed in connexion with hæmolysis by hæmolytic sera and snake venoms. If we adopt the view that after a certain ratio of amboceptor to complement has been reached the addition of increasing quantities of amboceptor brings about a condition whereby for every increment of amboceptor a certain amount of amboceptor and complement are rendered incapable of acting as such, then most of the observed phenomena are capable of interpretation by physico-chemical laws. If to a bacteriolytic serum we add increasing multiples of amboceptor it is conceivable that we might gradually bring about a state of affairs whereby all the complement was inactivated, or, falling short of this, in which the available complement was present in such high dilution that only certain organisms could make use of it in that dilution. In other words, by increasing the quantity of amboceptor we may produce a state of affairs comparable to that induced by absorption experiments with a time factor. If this be so, then we might expect that the phenomenon of diversion of the complement is not confined

⁷ Handbuch der Pathologischen Mikro-organismen (Kolle and Wassermann); Blood Immunity and Blood Relationship (Nuttall).

to immune sera—that is to say, that diversion of the complement should be produced by adding to a serum unit. (normal serum) steadily increasing quantities of the same serum heated. Further, if this be so it is obvious that by increasing the amount of immune body we are steadily increasing the dilution of the available complement, and so we could easily arrive at a stage where the dilution of the complement was such that the serum had ceased to be bactericidal for organisms requiring a relatively high concentration of complement but was still bactericidal for organisms requiring a much lower concentration of complement. We might thus obtain the false impression that this serum contained a series of specific complements instead of only one complement common to all. Experiments were conducted for the purpose of ascertaining (1) whether or not diversion of the complement can be induced in a normal serum by the addition of increasing quantities of the same serum heated; and (2) whether or not the diversion of the complement induced by adding increasing quantities of a heated immune serum is a specific action.

1. For the purpose of ascertaining whether or not diversion of the complement can be induced by the addition to a given quantity of normal serum increasing multiples of the same serum heated, the blood of a series of goats was tested. It was found, as might be expected, that considerable differences obtained between individual goats. In one goat, for example, it was found that the addition of an equal part of heated serum to the unheated serum produced marked loss of bactericidal power, whilst in other goats larger multiples were necessary before decrease of bactericidal action began to be apparent. In the earlier part of this paper I have noted that the goat which was used throughout these experiments only occasionally showed loss of bactericidal power when the quantity of amboceptor was trebled. The fact remains that diversion of the complement can be induced by the addition to a given unit of normal serum of increasing multiples of the same serum heated. In Table VII. an illustrative experiment is given.

TABLE VII.—Showing the Diversion of the Complement by adding Heated Normal Serum.

Dilutions of the 24 hours old living broth culture of bacillus typhosus employed as a test.	100c. mm. N.G.S.* 24 hours old (Goat 6) and 100 c.mm. of a dilution of the typhoid culture.	100 c.mm. N.G.S. 24 hours old (Goat 6), 100 c.mm. H.N.G.S.† (Goat 6), and 100 c.mm. of a dilution of the typhoid culture.
Undiluted culture	Growth	Growth
2 fold dilution	"	"
5 " " " " " "	"	"
10 " " " " " "	Sterile	"
25 " " " " " "	"	"
50 " " " " " "	"	"
100 " " " " " "	"	"
1,000 " " " " " "	"	Sterile
10,000 " " " " " "	"	"
100,000 " " " " " "	"	"
1,000,000 " " " " " "	"	"

* Normal goat's serum. Heated normal goat's serum.

2. To ascertain whether diversion of the complement is a specific action, two series of six tubes each were taken, the first two being small tubes of the type already described, the remaining four being ordinary sized test-tubes. I shall denote these two series as Series A and Series B respectively. Taking first Series A, the six tubes were charged as follows: Tube 1 received 50 cubic millimetres of sterile broth and 50 cubic millimetres of a 1 in 100,000 dilution of a 24 hours broth culture of bacillus typhosus; Tube 2, 50 cubic millimetres of normal goat's serum 24 hours old and 50 cubic millimetres of a 1 in 100,000 dilution of a 24 hours broth culture of bacillus typhosus; Tube 3, 50 cubic millimetres of normal goat's serum 24 hours old, 1500 cubic millimetres of normal salt, and 50 cubic millimetres of a 1 in 100,000 dilution of a 24 hours broth culture of bacillus typhosus; Tube 4, 50 cubic millimetres of normal goat's serum 24 hours old, 1500 cubic millimetres of heated normal goat's serum, and 50 cubic millimetres of a 1 in 100,000 dilution of a 24 hours broth culture of bacillus typhosus; Tube 5, 50 cubic millimetres of normal goat's serum 24 hours old, 1500 cubic

millimetres of heated typhoid immune serum, and 50 cubic millimetres of a 1 in 100,000 dilution of a 24 hours broth culture of bacillus typhosus; and Tube 6, 50 cubic millimetres of normal goat's serum 24 hours old, 1500 cubic millimetres of heated typhoid immune serum (cholera amboceptor free), and 50 cubic millimetres of a 1 in 100,000 dilution of a 24 hours broth culture of bacillus typhosus. The heated typhoid immune serum was obtained from a goat which had been immunised with the strain of bacillus typhosus used throughout these experiments. The words "cholera amboceptor free" mean that the cholera amboceptor had been removed from the heated typhoid immune serum. This was done as follows. The heated immune typhoid serum was digested for 24 hours at 37° C. with a dense sterilised emulsion of a 24 hours agar culture of cholera. The mixture was then centrifugalised and the clear fluid was pipetted off. With regard to Tubes 3, 4, 5, and 6, the mixture of normal goat's serum 24 hours old with normal salt, heated normal goat's serum, &c., was first made and the tubes were placed in the incubator for 15 minutes at 37° C., after which the 50 cubic millimetres of culture dilution were added to each tube. The tubes of Series B were prepared in precisely the same way with the exception that a 100,000-fold dilution of a 24 hours broth culture of cholera was used in place of a typhoid culture. All tubes of both series after having been prepared in the way stated were incubated at 37° C. for from 18 to 24 hours. At the end of that time 0.5 cubic centimetre of sterile broth was added to Tubes 1 and 2 of both series and six cubic centimetres of sterile broth to Tubes 3, 4, 5, and 6 of both series. All tubes were then incubated at 37° C. for 48 hours. In Table VIII. are set forth the constant results obtained throughout a long series of experiments extending over several months.

TABLE VIII.—Showing the Constant Results obtained in a Long Series of Experiments.
Cultures of the Bacillus Typhosus.

Dilution of the 24 hours old living broth culture of bacillus typhosus employed as a test.	50 c.mm. of sterile broth and 50 c.mm. of a dilution of the typhoid culture.	50 c.mm. N.G.S.* 24 hours old, and 50 c.mm. of a dilution of the typhoid culture.	50 c.mm. N.G.S. 24 hours old, 1500 c.mm. of normal salt, and 50 c.mm. of a dilution of the typhoid culture.	50 c.mm. N.G.S. 24 hours old, 1500 c.mm. H.N.G.S.† and 50 c.mm. of a dilution of the typhoid culture.	50 c.mm. N.G.S. 24 hours old, 1500 c.mm. H.T.I.S.‡ and 50 c.mm. of a dilution of the typhoid culture.	50 c.mm. N.G.S. 24 hours old, 1500 c.mm. H.T.I.S., cholera amboceptor free and 50 c.mm. of a dilution of the typhoid culture.
100,000 fold dilution	Growth	Sterile	Sterile	Growth	Growth	Growth

Cultures of the Spirillum Cholerae.

Dilution of the 24 hours old living broth culture of spirillum cholerae Asiaticae employed as a test.	50 c.mm. of sterile broth and 50 c.mm. of a dilution of the cholera culture.	50 c.mm. N.G.S. 24 hours old, and 50 c.mm. of a dilution of the cholera culture.	50 c.mm. N.G.S. 24 hours old, 1500 c.mm. of normal salt, and 50 c.mm. of a dilution of the cholera culture.	50 c.mm. N.G.S. 24 hours old, 1500 c.mm. H.N.G.S., and 50 c.mm. of a dilution of the cholera culture.	50 c.mm. N.G.S. 24 hours old, 1500 c.mm. H.T.I.S., and 50 c.mm. of a dilution of the cholera culture.	50 c.mm. N.G.S. 24 hours old, 1500 c.mm. H.T.I.S., cholera amboceptor free, and 50 c.mm. of a dilution of the cholera culture.
100,000 fold dilution	Growth	Sterile	Sterile	Sterile	Growth	Growth

* Normal goat's serum. † Heated normal goat's serum.
‡ Heated typhoid immune serum.

It will be seen that the addition in excess of heated normal goat's serum to a bacteriolytic mixture of the same serum unheated and a 100,000-fold dilution of a broth culture of typhoid or cholera was able to bring about diversion of the complement for typhoid but not for cholera. On substituting the heated serum of a goat immunised against typhoid fever we find, however, that the complement is diverted for both organisms. Further, when the cholera amboceptor has been

removed from this heated immune typhoid serum the result is still the same and the complement is invariably diverted for both organisms. Whatever explanation we may adopt of the phenomenon of "Komplementablenkung" these experiments show that it is certainly not specific, and therefore lends no support to the theory of the existence of specific complements. On the contrary, if this phenomenon can be used as an argument at all in this discussion, then it is strongly confirmatory of the experiments described in the first portion of this paper. At the outset it was stated that Longcope had failed to take into account the phenomenon of diversion of the complement and he performed no controls showing the bactericidal power of the re-activating quantity of normal serum used. It will be remembered that in the case of bacillus typhosus it was stated by Longcope that one-twentieth of a cubic centimetre of unheated serum was necessary to re-activate a cubic centimetre of heated serum. In these experiments I have shown that if 1.5 cubic centimetres of heated normal serum be added to one-twentieth of a cubic centimetre of the same serum unheated the bactericidal action of the serum for typhoid is entirely removed, the bactericidal action for cholera remaining unaltered. As the blood which Longcope was using was taken from patients suffering from typhoid fever it is by no means unlikely that the amboceptor content of their serum had undergone increase. It is therefore highly probable that the phenomenon which he attributed to specificity of the complement was in reality due to diversion of the complement by excess of immune body. Referring once more to these experiments on complement diversion I would submit that they can all be explained by the assumptions already set forth.

Summary.—1. Normal goat's serum 24 hours old in contact with clot is comparable with normal human serum in so far as its bactericidal action on bacillus typhosus and the cholera vibrio is concerned. 2. If normal goat's serum, the amboceptor content of which has been artificially increased, be saturated with a sterilised typhoid emulsion and incubated at 37° C. the dead typhoid bacilli are capable of removing from the serum all the complement for both bacillus typhosus and the cholera vibrio, provided sufficient time is allowed for the action. 3. If the process of absorption be stopped at a stage when the serum has lost its bactericidal power for bacillus typhosus but still remains bactericidal for cholera and the action again started the freshly added dead typhoid bacilli are still capable of removing the complement for cholera. 4. Diversion of the complement in normal serum may be induced by the addition of increasing quantities of the same serum heated. 5. This may give the appearance of multiplicity of complements. 6. If diversion of the complement be induced by the addition of heated immune typhoid serum diversion of the complement takes place for both typhoid fever and cholera, even though the cholera amboceptor has been removed from the heated immune typhoid serum.

Conclusions.—1. Normal goat's serum contains only one complement for cholera and typhoid fever. 2. The union between organism, amboceptor, and complement is probably dependent on degrees of concentration and upon a time factor.

I wish to express my gratitude to Lieutenant-Colonel D. Semple, R.A.M.C., Director of the Central Research Institute of India, who placed the resources of his laboratory at my disposal for this work, and to Captain G. Lamb, I.M.S., Director of the Pasteur Institute of India, and Captain W. F. Harvey, I.M.S., Deputy Sanitary Commissioner, Punjab, for much invaluable advice, assistance, and criticism. Kasauli, India.

WATER-SUPPLY OF TEIGNMOUTH.—A Local Government Board inquiry was recently held at Teignmouth, in Devonshire, to consider the application of the district council for sanction to borrow £21,830 for the purpose of obtaining a water-supply from the water-works of the Paignton district council, now in course of construction.

BUCKTHORN POISONING.—An inquest was held at Exeter on Nov. 10th concerning the death of an infant, aged two months. Evidence showed that the mother had given the baby some "buckthorn" after it had been vaccinated. It was stated that buckthorn was very commonly used in the neighbourhood. The jury returned a verdict of "Death from misadventure."