

ON HETEROLOGOUS AGGLUTININS MORE PARTICULARLY
THOSE PRESENT IN THE BLOOD SERUM OF CEREBRO-
SPINAL FEVER AND TYPHUS FEVER CASES.

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(1 Text Figure.)

IN previous papers Professor Symmers and myself have called attention to the presence of agglutinins for the typhoid bacillus, the colon bacillus, and a bacillus of the alcaligenes class in the blood serum of patients suffering from cerebro-spinal fever from whose lumbar puncture fluid and in some cases from whose blood the *Micrococcus intracellularis meningitidis* (Weichselbaum) had been cultivated.

The results obtained were so anomalous that it appeared to me desirable to continue the investigations and to find if our work could be brought into line with that of others in this special field. In consequence an extensive literature of the subject of Agglutination has been consulted and such facts as seemed to belong to the same class as ours noted.

In the present paper the subject matter has been arranged in the following order.

I. A brief recapitulation of the main points of the previous results obtained by Professor Symmers and myself.

II. A rapid survey of our knowledge of agglutination and in this way an endeavour is made to find the proper setting of our results.

III. In the concluding portion some further original observations are recorded.

PART I.

Agglutination of bacilli of the typhoid, colon and alcaligenes group by the blood serum of cases of cerebro-spinal fever.

Emulsions of agar cultures of the microorganisms were made in normal saline solution. The microscopic method of examination was used throughout and the results were recorded at the end of one or two hours.

Agglutination of the B. typhosus.

Seven out of 21 samples of the serum of cerebro-spinal fever patients examined "clumped" the *B. typhosus* in a 1 in 50 dilution, and of these seven three still gave a positive result with a 1 in 200 dilution and one with a 1 in 400 dilution.

Agglutination of the B. coli.

A "flaginac"¹ colon bacillus isolated from the faeces of a cerebro-spinal fever case was used as the test organism and 18 samples of blood serum were examined. In 16 of them the results were negative and in two positive with a 1 in 50 dilution of the serum. One of these cases still gave a positive reaction with a 1 in 400 dilution.

Agglutination of a bacillus of the alcaligenes class.

This bacillus is actively motile, forms a uniform emulsion in normal saline solution and in its growth on media resembles the *B. faecalis alcaligenes*. It was isolated from Belfast tap water. It was formerly called by us the *B. Grosvenor* as it was obtained from a house on Grosvenor Road; we now call it the *B. aquatilis alcaligenes*, and, bearing this name, it can be obtained from Professor Král, Prague.

We have examined altogether 184 different samples of the serum of cerebro-spinal fever cases and of these 153 gave a positive reaction with a dilution of 1 in 50. Of the 31 cases giving negative results 10 were found to be positive on a second examination, one on a third examination, three were examined several times and were still found negative, the remaining 17 were examined only once and it is probable that a later

¹ An organism producing fluorescence in glucose neutral red shake cultures, acid and gas in lactose broth, indol in peptone water, acid and clot in litmus milk.

examination of many of these would have given positive results ; in the case of others the examination was made late in convalescence when the agglutinins which were probably originally present had disappeared.

In round numbers 90% of the cases of cerebro-spinal fever agglutinated the *B. aquatilis alcaligenes* in a dilution of 1 in 50. In most cases agglutination still occurred with a 1 in 100 and higher dilutions. In 16 cases the blood serum gave marked "clumping" within an hour to dilutions of 1 in 1000, and, further, we found that one of the 16 agglutinated in 1 in 1400, 2 in 1 in 1500, 4 in 1 in 1600 and 4 in 1 in 2000 dilutions respectively. We have shown that it is possible to remove the agglutinins from the serum by saturation with the *B. aquatilis alcaligenes*, whilst saturation with the *Meningococcus*, *B. typhosus*, *B. coli communis* or *B. faecalis alcaligenes* (Král) leaves them intact. These results were controlled by the examination of the blood serum of healthy adults and of patients suffering from various infectious and general diseases.

Of the 31 specimens of the serum of normal adults examined 3, i.e. 9.6%, gave a positive reaction in a 1 in 50 and all a negative reaction in a 1 in 100 dilution of the serum. Of 137 samples of serum taken from patients suffering from various diseases 35 gave a positive and 99 a negative reaction in a 1 in 50 dilution, 3 a positive reaction in a 1 in 100 dilution. In higher dilutions than 1 in 100 all these cases gave negative results.

In round numbers 22% of the controls gave a positive result in a dilution of 1 in 50.

PART II.

Main facts in the Literature of Agglutination particularly those having reference to heterologous agglutination.

The discovery that the blood serum of an animal which had been immunised against certain microbes, e.g. *B. typhosus*, *B. cholerae*, etc., possessed a strong agglutinating action on the infecting microbe, soon proved to be a fact of great scientific and practical value. Gruber and Durham (1896) first applied "agglutinins" for the purpose of distinguishing microorganisms, whilst Grünbaum (1896) and Widal (1896) showed that in it clinical medicine had obtained a new method of diagnosing disease.

It was early recognised that the test was not a qualitative but a quantitative one. Stern (1903) as the result of an examination of the blood serum of normal adults showed that the reaction to be of value

must be obtained in a dilution of at least 1 in 40 of the serum. When the blood serum of typhoid fever patients was examined with regard to its agglutinative action on other bacilli, e.g. *B. coli*, *B. enteritidis*, it was found that these microbes also at times were agglutinated.

Some observers interpreted this fact as indicating that in typhoid fever not only was there an infection with the Eberth-Gaffky bacillus but also with certain intestinal microbes. The results obtained by observing the effect of the inoculation of animals with pure cultures showed that the increase of the agglutinins for *B. coli* in the blood of the animals which had been inoculated with the *B. typhosus* was due to the stimulating effect of the *B. typhosus* alone and in no way indicated a secondary infection with the *B. coli*. In this enquiry Pfaundler (1899), Durham (1900), Wassermann (1903), Jatta (1900), Jürgens (1903), Drigalski and Conradi (1903) took a prominent part. These researches impugned the specificity of the test. Pfaundler (1899) brought the knowledge at that time to a focus by saying that when an animal is immunised against an organism it produces in its serum not only agglutinins for this organism but also for nearly related microbes. In his own words specific agglutinins and group agglutinins are produced. Subsequent work showed that the agglutinative effects of an immune serum were increased not only for nearly related organisms but also for those which stood further away in a botanical classification. Wassermann (1903) brought the new facts into line concluding that in immunity primary agglutinins or hauptagglutinins are produced which act on the infecting organism and secondary or partialagglutinins which act on other organisms. This secondary or indirect action was called mitagglutination. The formation of partialagglutinins varied in different experiments. The particular strain of microorganism used, the species and also the individual peculiarities of the animals seemed to be factors in this variation.

Durham (1900) formulated a theory with regard to agglutinins which corresponded with Ehrlich and Morgenroth's discoveries as regards immune bodies. Like the latter, agglutinin was not to be considered as a single substance but consisted according to Durham of numerous single agglutinins which corresponded to the components of the agglutinogen substance of the bacteria. If one indicated the agglutinins with the letters, *A, B, C*, etc. and the agglutinogens with *a, b, c*, etc. the result of the possession of a number of similar agglutinogens was manifested in the phenomenon of mitagglutination. An example will make this clearer. The *B. typhosus* has, let us suppose, as its components *a, b, c, d, e*, the *B. enteritidis* *c, d, e, f, g, h*; the corresponding

sera possess the agglutinins *A, B, C, D, E*, and *C, D, E, F, G, H*. Typhoid immune serum works with all its single agglutinins *A, B, C, D, E*, on the corresponding components of the *B. typhosus* and gives therefore a maximal effect, while on *B. enteritidis* only the agglutinins *C, D, E* come into action and therefore mitagglutination is demonstrated only in low dilutions of the serum.

Clinical experience showed that in cases of jaundice very often the blood gave a positive Widal reaction with the *B. typhosus*. This phenomenon is explained as being due to infection of the biliary passages with either the *B. typhosus* itself or organisms which cause the formation of partialagglutinins for the Eberth-Gaffky bacillus.

In Weil's disease Eckardt (1902) and Zupnik (1902) found that the blood serum agglutinated the *B. typhosus*. In two cases of Eckardt's the reaction was positive even in a dilution of 1 in 1000. Eckardt regarded Weil's disease as a special form of infection with the *B. typhosus*; however Jäger's (1892) view that Weil's disease is a distinct entity and that the infecting microorganism is a pleomorphic member of the *proteus* group has received support from other investigators.

At this point some observations and experiments of Lubowski and Steinberg (1904) may be appropriately mentioned. These authors had two cases of mixed infection with *B. proteus* and *Staphylococci*. In both cases the infection arose from a suppurative otitis media. In the first case the blood serum of the patient agglutinated the *B. typhosus* and the *B. proteus* in dilutions of 1:40 and 1:80 respectively. In the second case the blood serum agglutinated the *B. proteus* isolated from the first case and the *B. typhosus* in dilutions of 1 in 320 and 1 in 40, later in dilutions of 1 in 2500 and 1 in 80 respectively. Thus there was an increase of the agglutinins during the course of the illness. Inoculation with Cholera bacilli and Streptococci was found to cause no production of agglutinins for the *B. typhosus* in the serum of the animals but when *Staphylococci* were employed the serum agglutinated the *B. typhosus* in a dilution of 1:640.

The saturation experiments of Bordet (1899), Eisenberg and Volk (1902) showed that in normal serum there are agglutinins for different organisms which can be removed by the corresponding organism and that this process leaves the agglutinin content for the other organisms unimpaired.

Another cause for secondary agglutinins in the blood is mixed infection. Castellani's (1902) application of the specific absorption

method appeared to solve the question whether the secondary agglutinins present in a blood serum were due to mitagglutination or to a mixed infection. Castellani claimed that saturation of the serum with the infecting microorganism removed not only its own agglutinins but also the partial or secondary agglutinins, whereas the latter remained unaltered if they were due to infection with their corresponding bacillus.

No doubt Castellani's experiment gives accurate results in many cases, especially those in which infection with typhoid and para-typhoid bacilli are concerned; still the experiments of Posselt and Sagasser (1903), Hetsch and Lentz (1903), Ballner and Sagasser (1904), Park and Collins, Symmers and Wilson (1907) seem to minimise its worth.

Posselt and Sagasser (1903) showed that in immunising there is not only an increase in the amount of agglutinins for the organism used but also of secondary or nebenagglutinins which act on other organisms. These nebenagglutinins as regards absorption behave like the special agglutinins in cases of mixed infection or as the agglutinins in normal serum. An example from their paper shows that sometimes these nebenagglutinins may be increased to a high degree. Thus the serum of a guinea-pig immunised against the *B. typhosus* and of a titre of 1:12000 for this bacillus had nebenagglutinins for *B. cholerae* 1:4500 and for *B. dysenteriae* 1:4000.

Hetsch and Lentz (1903) demonstrated by absorption with genuine cholera bacilli and cholera-like vibrios, the specificity of the agglutinins in normal horse serum and in that of an animal immunised against the *B. cholerae*. Saturation with *B. cholerae* diminished the agglutinins for this organism whilst the nebenagglutinins remained either the same or were but slightly diminished.

Ballner and Sagasser (1904) showed that a homologous bacterial species can withdraw from an immune serum only its own agglutinins but not the nebenagglutinins which act on other bacteria: also a heterologous bacterial species binds only its own partial agglutinins and no other portion of the total agglutinins, so that for this reason Ballner and Sagasser conclude that the absorption of agglutinins through homologous and heterologous microorganisms must be regarded as a strong specific reaction. Examples given in Ballner and Sagasser's paper show that the nebenagglutinins are at times markedly increased, that inoculation with the *B. tetani* and *B. pneumoniae* of Friedlander lead to the formation of few hauptagglutinins but numerous nebenagglutinins.

Park and Collins (1908) showed (1) that the group agglutinins may be enormously increased, (2) that injection of bouillon alone may increase the agglutinins in a horse's serum, (3) that the absorption method simply proves that when one variety of bacteria removes all agglutinins for a second the agglutinins under question were not produced by that second variety.

Collins (1908) has recently shown that inoculation with various non-bacterial bodies (e.g. enzymes, yeasts, simple salts containing either a sulphur or phosphorus atom) leads to the formation of agglutinins for dysentery bacilli.

It is well known that Ehrlich's Theory of Immunity implies the presence of protective substances already existing in the body and that in the immunising process these are increased in amount.

How the agglutinins of normal serum come into existence is obscure. The work of Grünbaum (1897), Müller (1904), Pfaundler, Kraus and Löw (1897) and Schumacher (1901) shows that the agglutinins for *B. coli* and *B. typhosus* are either absent or present in only very small amounts in the blood of young children and animals. In other words the agglutinating power of normal serum is not inborn but is produced during life. One may suppose a process of immunisation to be going on continually, the organisms in the intestine causing the formation of agglutinins in the blood. It may be noted that Fraenkel and Otto (1897) succeeded in increasing enormously the agglutinins in dog's blood by feeding the animals with large doses of typhoid bacilli.

The explanation of the fact that the blood serum of Europeans contains specifically absorbable agglutinins for the *B. cholerae* and the *B. pestis* may be that these agglutinins are partialagglutinins or nebenagglutinins caused by the action of unknown saprophytic intestinal organisms.

It is not unlikely that saprophytic organisms resembling pathogenic ones are in the alimentary tract and that hauptagglutinins are produced for these organisms and nebenagglutinins for their pathogenic relations. It is well known that agglutinins can be formed by inoculation with the simplest saprophytes, e.g. *B. mesentericus*.

Whether the agglutinins of normal and immune serum are identical is undecided. Ehrlich's theory supposes that immunisation is only the normal production of receptors carried to excess.

Landsteiner and Reich (1908) in a recent paper have pointed out that the haemagglutinins of normal serum are more easily removed than the homologous agglutinins of immune serum by shaking the serum with casein. Normal agglutinins were also found to be more heat

labile than immune ones. They also showed that each single agglutinin of normal serum has an affinity for a number of different corpuscles and that the amount of this affinity varied in different cases. They remark "in the language of Ehrlich our results tend to the view that the haptophorous groups of normal agglutinins are so composed that they can react with the different receptors of very many blood corpuscles but in unlike degree. But according to Ehrlich's theory each haptophorous group ought to react with only a single fixed kind of receptor." They also found that when an animal was inoculated with the blood corpuscles of another species not only the homologous agglutinins but also the *neben-agglutinins* which act on the blood corpuscles of other species increase. These results correspond with those of Posselt and Sagasser and Hetsch and Lentz.

As a rule the homologous agglutinins relatively preponderated but to this in two cases there were striking exceptions, in which during the immunising the heterologous agglutinins became very high and were distinctly higher than the homologous ones.

The very definite results obtained by absorption experiments point to the presence in normal serum of specific agglutinins for the different bacteria. The recent work of Bürgi (1907), Mamlok (1908) and Hirschfeld (1907) has furnished results rather opposed to this view and which find their simplest explanation in the assumption of a single normal agglutinin which acts on all bacteria and blood corpuscles.

Bürgi (1907) found (1) that if a normal serum agglutinated one bacillus strongly or weakly, it had a proportionately strong or weak effect on other bacteria, (2) that animals could be arranged in a series according to the action of their blood serum on the most different bacteria. Cattle serum had the strongest whilst guinea-pig serum had the weakest agglutinative effect.

From this review which I shall conclude with a quotation from Paltauf it can be seen that the results of Posselt and Sagasser, Hetsch and Lentz, Ballner and Sagasser, and Park and Collins are along the same lines as those obtained by Professor Symmers and myself.

Paltauf (1904) says the results of Posselt and Sagasser as well as those of Hetsch and Lentz go to show that in the immune serum of animals as well as in that of sick men and women heterologous agglutinins exist which have no binding groups for the infecting bacteria but are as specific as regards absorption as those developed in a mixed infection. They must therefore be distinguished from partial agglutinins or *mitagglutinins*. They can be designated as "heterologous *neben-*

agglutinins" or more briefly as nebenagglutinins. For their formation the views held regarding partialagglutinins do not apply.

To explain their origin one must assume that besides the receptors (homologous) that have binding groups fitted to the agglutinogens of the infecting organism other closely related receptors are set free. In part they would appear to be normal agglutinins whose production through an adequate stimulus is increased.

Perhaps one may conceive of them being fixed to the same protoplasm as that possessing the homologous receptors.

We have moreover a few examples of a receptor apparatus being stimulated to secretion through the irritation of a non-homologous haptophorous group. In this connection one may recall Verney's observations on the mutual influence of consecutive immunisations which showed that immunisation with typhoid bacilli affected the agglutinins for the *B. coli*.

Most pertinent are the observations of Obermeyer and Pick (1904) regarding the formation of a heterologous precipitin after it had been once formed through homologous immunisation and then a foreign protein injected. They saw the precipitins which had been formed as the result of inoculation several months previously with cattle serum and which had disappeared reappear on the injection of horse albumoses, and in such amounts that they could not be regarded as partialprecipitins.

We may also mention that v. Dungern (1903) observed among rabbits injected with majaplasma one the serum of which precipitated not only majaplasma but also octopusplasma.

The explanation of the production of heterologous agglutinins may be that infection with certain germs leads to an alteration of the bacterial flora of the intestine. As the result of this secondary auto-infection, along with the agglutinins for the primary infecting organism, agglutinins are formed for the intestinal microorganisms also.

PART III.

The explanation of the presence of these agglutinins for the *B. typhosus*, *B. coli* and *B. aquatilis alcaligenes* in the blood serum of patients infected with Weichselbaum's Meningococcus is either that there is a mixed infection or that the agglutinins are heterologous nebenagglutinins, the latter term being used to indicate agglutinins which are

shown by the absorption test to be distinct from the meningococcal agglutinins.

Against the idea of a mixed infection are the facts—(1) that no bacilli resembling the *B. typhosus* or the *B. aquatilis alcaligenes* were ever obtained from the blood, urine or organs of the cases though these were frequently examined by cultural methods, (2) that we got similar results with the blood serum of Scottish cases—we may recall the fact that the *B. aquatilis alcaligenes* was isolated from Belfast tap water, (3) that, as will be shown later, immunisation of animals with the Meningococcus increases the agglutinins for the *B. aquatilis alcaligenes*, (4) that the phenomenon was observed in such a large number of cases as to render the hypothesis of a double infection extremely improbable.

In this Part experiments were carried out to determine:

(1) the course of development of the agglutinins for the Meningococcus, and for the *B. aquatilis alcaligenes* in a case of cerebro-spinal fever;

(2) the effect of heat on these agglutinins;

(3) with what component of the serum the agglutinins for the *B. aquatilis alcaligenes* are bound;

(4) whether immunising an animal with the Meningococcus increased the agglutinins for the *B. aquatilis alcaligenes* in its blood and *vice versa*.

Development of agglutinins during the course of Cerebro-spinal Fever.

The blood serum of a girl 8 years of age was examined at short intervals as to its agglutinative action on a strain of the Meningococcus and on our culture of the *B. aquatilis alcaligenes*. The case was under observation from 30 hours after the onset of the disease until 47 days later at which time the patient left hospital completely cured.

The experiments were carried out at room temperature with emulsions in normal saline solution of agar and ascitic agar cultures of the *B. aquatilis alcaligenes* and Meningococcus respectively.

The results were recorded at the end of 2 hours. In Table I +++ indicates very large clumps, ++ clumps of moderate size, + small clumps.

From these results we see that in this case there was a rapid increase in the agglutinins for the Meningococcus and for the *B. aquatilis alcaligenes* during the fourth day of the disease, that these both attained

TABLE I.

Name of patient	Date	Day of disease	Meningococcus Strain G					E. agglutinis abaligines									
			Dilution of serum					Dilution of serum									
J. M.			10	15	20	30	40	20	40	60	80	100	200	300	400	500	
	7/4/09	2nd day (30 hrs.)	-	-	-	-	-	+	-	-	-	-	-	-	-	-	
	9/4/09	4th day	++	-	-	-	-	++	++	++	+	-	-	-	-	-	
	11/4/09	6th "	++	++	+	-	-	++	++	++	++	++	++	+	-	-	
	13/4/09	8th "	++	++	++	++	++	++	++	++	++	++	++	++	+	-	
	15/4/09	10th "	++	++	++	++	++	++	++	++	++	++	++	++	++	-	
	17/4/09	12th "	++	++	++	++	+	++	++	++	++	++	++	++	-	-	
	19/4/09	14th "	++	++	++	++	+	++	++	++	++	++	++	++	-	-	
	21/4/09	16th "	++	++	++	++	+	++	++	++	++	++	++	++	-	-	
	23/4/09	18th "	++	++	++	+	-	++	++	++	++	++	++	++	-	-	
	29/4/09	24th "	++	++	++	+	-	++	++	++	++	++	++	++	-	-	
	3/5/09	28th "	++	++	+	-	-	++	++	++	++	++	++	++	-	-	
	7/5/09	32nd "	++	+	-	-	-	++	++	++	++	++	++	++	-	-	
	15/5/09	40th "	++	-	-	-	-	++	++	++	++	++	++	++	-	-	
	23/5/09	48th "	-	-	-	-	-	++	++	+	-	-	-	-	-	-	

their maximum on the eighth day, after which date the meningococcal agglutinins declined slowly and the agglutinins for the *B. aquatilis alcaligenes* more rapidly.

When the agglutination results for these two organisms are plotted out in the form of curves it is seen that the latter are to a certain extent parallel. From many other experiments we have an impression that as a general rule a serum possessing a high content of agglutinins for the Meningococcus has also a high content for the *B. aquatilis alcaligenes*. This however is by no means invariably the case as can be seen in Table II.

TABLE II.

Name of patient	Date	Day of disease	Meningococcus strain G								<i>B. aquatilis alcaligenes</i>			
			5	10	20	40	50	60	80	20	40	60	100	
S.	8/2/09	3rd	+++	+++	+++	++	+	-	-	-	-	-	-	
	11/2/09	6th	+++	+++	+++	++	+	-	-	-	-	-	-	
	18/2/09	13th	+++	+++	+++	+++	++	+	-	+++	+++	+	-	
	11/3/09	34th	+++	+++	+++	+++	++	+	-	++	-	-	-	

Table II also shows that agglutinins for the *B. aquatilis alcaligenes* may develop during the disease and yet a single examination of the blood may show no indication of them.

With reference to the agglutination of Meningococci it may be of interest to record here a result we obtained which showed that a great difference as regards agglutination exists between strains of Meningococci obtained from epidemic and sporadic cases.

Agglutination of Meningococcus (Strain D, epidemic).

	10	20	40	100	200	300	400	600	800
Flexner's Serum No. I	+++	+++	+++	+++	+++	+++	++	-	-
Ruppel's Serum No. II	+++	+++	+++	+++	++	+	-	-	-

Agglutination of Meningococcus (Sporadic Strain).

	10	20	40	100	200	300	400	600	800
Flexner's Serum No. I	-	-	-	-	-	-	-	-	-
Ruppel's Serum No. II	-	-	-	-	-	-	-	-	-

Effect of heat on the agglutinins for the Meningococcus and for the *B. aquatilis alcaligenes*.

This was tested by noting the effects of heating a specimen of a cerebro-spinal fever patient's serum and a specimen of a commercial antimeningococcal serum at 60° C. and at 65° C. for 10 minutes.

The results are represented in Table III in which +++ denotes large, ++ moderate sized and + small clumps, whilst - indicates absence of agglutination.

TABLE III.
Meningococcus (Strain G).

	Dilutions of serum							
	2	5	10	20	30	40	60	100 200
Patient's Serum :								
Before	+++	+++	+++	+++	++	+	-	- -
After 10 mins. at 60° C.	-	-	-	-	-	-	-	- -
After 10 mins. at 65° C.	-	-	-	-	-	-	-	- -
Antimeningococccic Horse Serum :								
Before	+++	+++	+++	+++	+++	+++	+++	++ -
After 10 mins. at 60° C.	-	-	-	-	-	-	-	- -
After 10 mins. at 65° C.	-	-	-	-	-	-	-	- -

B. aquatilis alcaligenes.

	Dilutions of serum							
	20	40	60	100	200	300	400	500 600
Patient's Serum :								
Before	+++	+++	+++	+++	+++	+++	++	++ -
After 10 mins. at 60° C.	+++	+++	+++	++	-	-	-	- -
After 10 mins. at 65° C.	-	-	-	-	-	-	-	- -
Antimeningococccic Horse Serum :								
Before	+++	+++	+++	+++	+++	++	+	- -
After 10 mins. at 60° C.	+++	++	+	-	-	-	-	- -
After 10 mins. at 65° C.	-	-	-	-	-	-	-	- -

It is evident from above Table that heating the serum for 10 minutes at 60° C. completely destroys the agglutinins for the Meningococcus but only partially those for the *B. aquatilis alcaligenes*, and that heating for 10 minutes at 65° C. totally destroys the agglutinins both for the Meningococcus and for the *B. aquatilis alcaligenes*.

With what component of the serum are the agglutinins for the *B. aquatilis alcaligenes* bound?

The original titre of a serum was as follows :—

100	500	800	1000
+++	+++	+++	++

Equal volumes of this serum and of a saturated watery solution of ammonium sulphate were taken. A precipitate of globulin was formed. The mixture was centrifugalised and the supernatant fluid was found to be devoid of agglutinative effect. The precipitate was dissolved in normal saline solution and the volume made up to that of

the original volume of the serum. This solution was found to agglutinate the *B. aquatilis alcaligenes* in the following manner:—

100	200	500	800	1000
+++	+++	+++	++	+

To discover what fraction of the agglutinin was bound up with that portion of the globulin known as the "euglobulin" and what with the "pseudoglobulin" component, the following experiment was performed.

2.5 c.c. serum, 5.75 c.c. distilled water and 4.25 saturated watery solution of ammonium sulphate were mixed together so that there were 3.4 c.c. of saturated watery solution of ammonium sulphate in 10 c.c. After standing two hours at room temperature the mixture was centrifuged. The supernatant fluid contained the "pseudoglobulin" fraction. The precipitate (euglobulin) was washed with water containing 3.4 c.c. saturated ammonium sulphate solution per 10 c.c. The "euglobulin" was dissolved in normal saline solution and the volume made up to 2.5 c.c.

The agglutination titre of the pseudoglobulin and euglobulin fractions expressed in terms corresponding to the original serum was then found to be:—

	100	200	300	400	500	800	1000
Pseudoglobulin	+++	+++	+++	+++	+++	++	+
Euglobulin	++	-	-	-	-	-	-

Table IV concisely explains the results of this experiment.

TABLE IV.

	Dilutions of serum				
	100	200	500	800	1000
Original Serum	+++	+++	+++	+++	++
Total Globulin	+++	+++	+++	++	+
Pseudoglobulin	+++	+++	+++	++	+
Euglobulin	++	-	-	-	-

+++ indicate large, ++ moderate sized, + small clumps, - a negative reaction.

Does immunising an animal against the Meningococcus increase in its blood the agglutinins for the *B. aquatilis alcaligenes*?

Two rabbits were inoculated with cultures of the Meningococcus at intervals of 10 days for three months. At the end of this time the agglutinins for the Meningococcus were but slightly increased and those for the *B. aquatilis alcaligenes* not at all.

One of the rabbits was now given two inoculations with the

B. aquatilis alcaligenes as the result of which its blood serum agglutinated this bacillus in a dilution of 1 in 2000 in $\frac{1}{2}$ hour but the agglutinins for the Meningococcus were not increased.

The action of various antimeningococcic sera was now tested on the Meningococcus (strain G) and on the *B. aquatilis alcaligenes*.

The results obtained are expressed in Table V.

TABLE V.

Test organisms	Dilutions of serum									
	5	10	20	40	60	80	100	200	300	400 500
serum No. I a:										
Meningococcus (Strain G)	+++	+++	+++	+++	+++	+++	++	-	-	-
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+++	+++	+++	+++	+++	++	-
serum No. I b:										
Meningococcus (Strain G)	+++	+++	+++	+++	+++	+++	+++	+	-	-
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+++	+++	+++	+++	+++	++	-
serum No. II:										
Meningococcus (strain G)	-	-	-	-	-	-	-	-	-	-
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+	-	-	-	-	-	-
serum No. III:										
Meningococcus (Strain G)	-	-	-	-	-	-	-	-	-	-
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+	-	-	-	-	-	-
three specimens of normal horse serum:										
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+++	++	+	+	-	-	-
" "	+++	+++	+++	+++	+++	++	++	-	-	-
" "	+++	+++	+++	+++	++	+	+	-	-	-

These results although not convincing tend to show that when a horse has been immunised against the Meningococcus and has agglutinins for this organism present in its serum that along with them agglutinins for the *B. aquatilis alcaligenes* are produced in excess.

Does immunising an animal against the *B. aquatilis alcaligenes* produce in its blood agglutinins for the Meningococcus?

A rabbit was inoculated subcutaneously on two occasions with two agar cultures of the *B. aquatilis alcaligenes*.

Before the first inoculation the agglutinative action of its blood serum on the Meningococcus and on the *B. aquatilis alcaligenes* was as follows:—

	Dilutions of serum			
	2	5	10	20
<i>B. aquatilis alcaligenes</i>	-	-
Meningococcus (Strain G)	-	-	-	-

At the end of 20 days the agglutination titre was found to be

	2	5	10	20	500	1000	1500
<i>B. aquatilis alcaligenes</i>	+++	+++	+	-
Meningococcus (Strain G)	-	-	-	-	-	-	-

We conclude then that in the rabbit at any rate inoculation with *B. aquatilis alcaligenes* does not produce in its blood serum agglutinins for the Meningococcus. We may note that the rabbit which has been inoculated with Meningococci and then received two injections of the *B. aquatilis alcaligenes* seemed to form more agglutinins for the *B. aquatilis alcaligenes* than the rabbit which received the same number of injections of the *B. aquatilis alcaligenes* alone.

An interesting and practical question arises here "Does inoculation of an animal with cultures of the Meningococcus and of the *B. aquatilis alcaligenes* lead to the production of a more powerful antimeningococccic serum than that produced when the Meningococcus alone is used?" We can give no answer to this question at present.

EXAMINATION OF THE BLOOD SERUM IN CASES OF TYPHUS FEVER.

Agglutinative action on the B. typhosus.

Thirty-one different samples of the blood serum obtained from Belfast cases of typhus fever were examined as to their action on the *B. typhosus*, with the result that 18 gave a positive and 13 a negative reaction when a 1:50 dilution of the serum was used.

On several occasions different strains of typhoid bacilli were employed and almost invariably specimens of blood from non-typhus cases were simultaneously examined with reference to the Widal test, and so these served to control our results.

In two cases there was still a trace of agglutination with a 1:200 dilution. In a few of the cases there was a history of a previous attack of typhoid fever but in the majority there was no such history, and in one of the cases (a young girl) in which the serum gave a positive reaction in a dilution of 1:200, minute enquiry failed to elicit any account of a preceding illness.

Agglutination of a bacillus isolated from the stools of a typhus fever case.

On smearing a loopful of faeces obtained from a typhus fever case over a Conradi-Drigalski plate red and blue colonies in equal

number developed. On subculture the blue colonies proved to be composed of bacilli having the following characters.

Morphology:—bacilli very similar in appearance to typhoid bacilli but non-motile,—Gram-negative.

Cultural characters:—the growth on agar and gelatin media is similar to that of the *B. typhosus*. On gelatine plates it forms transparent “vine leaf” surface colonies:—no liquefaction of the gelatin results.

Bouillon and peptone water:—uniform turbidity. Well marked indol reaction at the end of four or five days.

Potato:—whitish almost invisible growth.

Litmus milk first becomes slightly acid, then more alkaline, finally becomes acid again and clots. The action of this bacillus on lactose is peculiar. On solid media containing this substance (e.g. that of Conradi-Drigalski) the colonies are blue, there being no evidence of acid production. In lactose litmus broth no change beyond the production of a uniform turbidity is visible until the fifth day when the medium becomes slightly acid, later the acidity becomes more marked and finally gas is also produced.

Glucose, mannite, dulcitate and arabinose are fermented with the production of acid and gas.

Raffinose shows no change.

It is evident from the above description that this organism, which for convenience of reference we shall name the *Bacillus U*, is a variant strain of the *B. coli*.

When first isolated the *Bacillus U* was agglutinated by normal serum in a 1:60 but not in a 1:100 dilution. A sample of typhus serum agglutinated it at this time in a 1:300 dilution. After a few subcultures normal serum agglutinated the bacillus slightly with a 1:300 dilution whilst the effect of typhus serum was proportionately increased.

In Table VI is shown the agglutinative action of the blood serum of seven typhus fever cases (the only ones examined) and of three normal adults on this bacillus.

From a consideration of this Table it is evident that the blood serum of typhus fever cases has on an average five times the agglutinative action of normal serum on the *Bacillus U*.

I may add that these patients were undoubtedly suffering from typhus fever. The clinical picture was typical and in nearly all cases many members of the same family were attacked. Moreover the blood

was examined by cultural methods in 32 cases and the flasks either remained sterile or there was a growth of Diplococci but never of bacilli.

We never obtained the *Bacillus U* from any part of the body except the intestine. The fact that the *Bacillus U* was never obtained from the blood would seem to show that it had no etiological connection with the disease.

TABLE VI.

Name	Dilutions of serum employed							
	100	200	300	400	500	600	800	1000
F. McC., 4 wks. convalescent	+++	+++	+++	+++	+++	+++	++	++
E. U. „ „	+++	+++	+++	+++	+	-	-	-
H. U. „ „	+++	+++	+++	+++	+++	+++	++	++
S. U. „ „	+++	+++	+++	+	-	-	-	-
N. T. „ „	+++	+++	+++	+++	+++	+++	+++	++
M. C. „ „	+++	+++	+++	+++	+++	+++	+++	+++
B. „ „	+++	+++	+++	+++	+++	+++	+++	+++
R. M. S., normal	+++	+++	+	-	-	-	-	-
W. J. W. „ „	+++	+++	+	-	-	-	-	-
T. H. „ „	+++	+++	+	-	-	-	-	-

Name	Dilutions of serum employed							
	1200	1400	1600	1800	2000	3000	3500	4000
F. McC., 4 wks. convalescent	++	+	+	-	-	-	-	-
E. U. „ „	-	-	-	-	-	-	-	-
H. U. „ „	-	-	-	-	-	-	-	-
S. U. „ „	-	-	-	-	-	-	-	-
N. T. „ „	++	+	-	-	-	-	-	-
M. C. „ „	++	+	+	-	-	-	-	-
B. „ „	+++	+++	+++	+++	+++	+++	++	++
R. M. S., normal	-	-	-	-	-	-	-	-
W. J. W. „ „	-	-	-	-	-	-	-	-
T. H. „ „	-	-	-	-	-	-	-	-

After we had made the above observations on the action of the blood serum of typhus fever on the *Bacillus typhosus* and on the *Bacillus U* we read with interest a paper by Dr T. Horiuchi (1908).

This observer made a bacteriological study of 40 cases of a fever which occurred amongst the Japanese troops during the Russo-Japanese War. From the faeces of some of the patients he cultivated a bacillus on which the blood serum of the cases had a high agglutinative effect. Culturally his bacillus is identical with the *Bacillus U* described by me. In three cases Horiuchi obtained the same bacillus from the urine but the blood of 40 cases although examined daily gave no growth.

The fever ran a course like typhus but whether it was genuine typhus fever was left an open question. Horiuchi calls the disease "Febris exanthematicus Mandschurici" and his bacillus "Bacillus febris exanthematici Mandschurici." He also found that in some cases the blood serum agglutinated the *B. typhosus* and regarded this as an instance of a group reaction.

The fact that the blood serum of typhus fever cases in Manchuria and in Ireland should have been independently discovered to have an agglutinative action on an intestinal organism is rather interesting, but whether the phenomenon should be taken as an instance of specific or of heterologous agglutination we must leave for the present undecided.

These observations show that the Widal test cannot be relied upon to distinguish typhus from typhoid fever.

Those who have much experience in the use of this test recognise its great value but they also know that occasionally a positive result is obtained with the blood serum of patients whom clinical and pathological evidence eventually proved not to have been infected with the *B. typhosus*.

We shall briefly refer to a few striking instances of this, mentioning the names of the observers, the diagnosis and the dilution of the serum employed in the test.

(1) In cases of infection with staphylococci and streptococci:—

Megele (1903). A case of liver abscess from the pus of which a staphylococcus aureus was obtained in pure culture.

Heinrich Gräf (1906). A case of streptococcal septicaemia. Dilution of serum 1 in 100.

Lommel (1902). A case of puerperal fever. 1 : 80.

Hale White and Pakes (1902). A case of streptococcal endocarditis. 1 : 200.

(2) Pneumonia:—

Kassel and Mann (1899). Two cases of croupous pneumonia. 1 : 50.

Jul. G. Iversen (1905) gives details of a very interesting case. This was a patient 21 years of age. The agglutination test was six times employed. On the 11th and 13th days of the disease there was no agglutination with a 1 : 50 dilution. On the 16th and 19th days the reaction was positive with 1 : 500 dilution. On the 20th day clumping still occurred with a 1 : 1500 dilution and the same result was obtained with the serum taken at the autopsy. The post-mortem examination

revealed diphtheria bacilli and Streptococci in the throat and Streptococci were also cultivated from the spleen, liver and tonsils. The lungs showed a croupous pneumonia with Fraenkel's Pneumococcus in the exudate. There was no evidence either anatomical or bacteriological in favour of infection with the *B. typhosus*. There was a slight catarrhal enteritis.

(3) In Meningitis:—

Van Oordt (1897). A case of pneumococcal endocarditis and meningitis. 1:40.

Jez (1897) and Marcuse (1908). Cases of tubercular meningitis. 1:100.

Symmers and Wilson (1907). A case of meningococcal meningitis. 1:200.

(4) In Tuberculosis:—

Jul. G. Iversen (1905). Three cases of pulmonary tuberculosis giving positive reactions with dilutions of 1:250, 1:250 and 1:50 respectively.

Ernest Krencker (1908) examined the serum of 26 cases of tuberculosis and found that eight cases gave a positive reaction with a 1:50 dilution and of these eight, three were still positive with a 1:200 dilution.

CONCLUSIONS.

1. The results obtained (see *Journ. of Hyg.* 1908, Vol. VIII. No. 3, p. 313) as to the agglutinative action of the blood serum of cerebro-spinal fever cases on the *B. typhosus*, *B. coli* and *B. aquatilis alcaligenes* are confirmed and extended.

2. The general setting of these results with regard to the main facts in the literature of agglutination is undertaken and the conclusion is reached that these secondary agglutinins do not indicate a mixed infection but are of the nature of heterologous "Nebenagglutinine." If this is so the observations of Professor Symmers and myself are of a similar nature to those recorded by Posselt and Sagasser, Hetsch and Lentz, Ballner and Sagasser and Park and Collins.

3. The course of development of the agglutinins for the Meningococcus and for the *B. aquatilis alcaligenes* in the blood of a case of cerebro-spinal fever was followed from day to day. When the results obtained were represented by curves a certain degree of parallelism between the latter was found to exist.

4. The heterologous agglutinins are bound up with the globulin component of the serum: the greater part belongs to the "pseudoglobulin," a smaller part to the "euglobulin" fraction.

5. Heating the serum at 60° C. for 10 minutes destroys completely the agglutinins for the Meningococcus but only partially those for the *B. aquatilis alcaligenes*. Heating at 65° C. for 10 minutes completely destroys the agglutinins both for the Meningococcus and for the *B. aquatilis alcaligenes*.

6. The blood serum of 18 out of 31 cases of typhus fever examined was found to agglutinate the *B. typhosus* in 1:50 dilution. The Widal test cannot be relied on to distinguish typhoid from typhus fever. Blood cultural methods are preferable.

7. Seven cases of typhus fever were examined as to their action on a bacillus which had been isolated from the stools of one of the cases. On an average the fever cases had five times the agglutinative action of normal serum.

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