THE CONGLUTINATION PHENOMENON

I. AN INTRODUCTION TO THE CONGLUTINATION PHENOMENON AND AN ACCOUNT OF THE OBSERVATIONS AND VIEWS OF PREVIOUS INVESTIGATORS

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CONTENTS

I. INTRODUCTION

The present research on the conglutination reaction was begun in 1941 when one of the authors (N.H.H.) was directed to carry out investigations as a precautionary measure on the diagnosis of glanders. It was decided to compare the so-called 'Conglutination Test', so long associated with the serodiagnostic procedures, which may be used in this condition, with the haemolytic complement fixation test. The favourable results obtained stimulated further investigation of the conglutination phenomenon.

The name conglutination suggests 'the act of gluing together', and was the name given by Bordet & Streng in 1909 to describe the clumping of red blood cells previously sensitized with an antibody, and which had also adsorbed complement, by a substance called conglutinin, existing in the serum of normal bovines. The fact that the action both of complement and conglutinin was required in conglutination clearly differentiated it from the more simple process of agglutination.

Streng (1909*b*) demonstrated conglutination with bacteria instead of red blood cells. He stated that the reaction was specific in that it required the intermediary action of an immune body, and suggested its use as a serological procedure, because it would detect sensitization by an immune body in itself too weak to cause agglutination. This method of applying the principle of the conglutination phenomenon in order to enhance the activity of a specific antibody by adding to the antigen-antibody system a source of complement and conglutinin we have called the *Direct Conglutination Reaction.*

In 1910 Streng, bearing in mind that complement was necessary for conglutination, developed a complement absorption test on similar lines to the Bordet-Gengou complement fixation test. The complements used in the conglutination reaction are those existing in the fresh sera of certain species, such as the horse, pig and cat. These complements are not normally haemolytic unless used in very high concentrations. Whether it is correct to use the term complement for such a substance which is adsorbed by sensitized cells, but which is not haemolytic, will be discussed later. In this complement absorption test the conglutinating complement is first mixed with the antigen-antibody system, and after a certain time a conglutinating system, consisting of sheep cells, antibody to sheep cells, and conglutinin, is added in order to test for free complement. If complement has not been adsorbed in the first stage, it will clump or conglutinate the sheep cell indicator system. We have called this serological procedure the *Conglutinating Complement Absorption Test* to indicate the analogy to and, at the same time, to differentiate it from the usual haemolytic complement fixation test. The use of the term 'Conglutination Reaction' for this test is misleading, as in the past it has been used indiscriminately to describe both direct conglutination and the conglutinating complement absorption test.

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It may seem that this conglutinating complement absorption test does not differ very much from the ordinary haemolytic complement fixation test. The principle certainly is the same, but the two reactions differ in the nature of the complement used. 'Non-haemolytic' complements are used in the former reaction, whilst a 'haemolytic complement' is essential for the latter.

Usually when serologists talk of complement they infer guinea-pig complement, which, as stated, is essentially a haemolytic complement. However, to confine the term complement to a complement complex which is haemolytic would hardly seem, in our opinion, to be correct. It would appear that the definition of complement is determined not by its haemolytic action (as many reagents are haemolytic) but rather by its property of being adsorbed by a sensitized antigen, and not by an unsensitized one. Before such a definition can be accepted it is necessary that the adsorption of a complement be demonstrated by some observable reaction. In the case of red cells the adsorption of guinea-pig 'haemolytic complement' is demonstrated by haemolysis; adsorption of 'non-haemolytic' complement can, on the other hand, be directly demonstrated by conglutination. Cells which have adsorbed a 'non-haemolytic' complement are not lysed but are clumped or conglutinated in the presence of conglutinin.

Guinea-pig complement is described as being composed of components 1, 2, 3 and 4—all of which must be present in sufficient amounts if the complex is to be haemolytic. The fresh sera of all animals contain certain components of this complement complex in varying amounts. Many of these complexes, although not haemolytic, exhibit complement activity, in that they are adsorbed on to sensitized cells, which can then be clumped together by the action of conglutinin.

Conglutinin has no action on normal, or even sensitized, cells but acts only on sensitized cells which have also adsorbed a conglutinating complement.

With this brief introduction, we would like to state a few of our reasons for considering that a reinvestigation of this phenomenon may well prove of value.

As already mentioned the conglutinating complement absorption test has similar applications to those of the haemolytic complement fixation test. The main difference between the two reactions lies in the complement used. We are of the opinion that the choice of complement for a particular immune system may be a matter of great importance. It is well known that the properties of an antibody vary considerably, depending on the species of animal from which it is derived. In the same way it seems likely that the degree to which a complement is adsorbed depends on the species nature of the proteins, and that different immune aggregates adsorb the complement proteins of one species in preference to those of another. The complement most easily adsorbed by a certain system may well be one which is not haemolytic, and thus can only be detected by the conglutination reaction.

The sera of certain animals, such as those of the mule and donkey, may be very anticomplementary to guinea-pig complement, so much so that the haemolytic reaction cannot be used. These same sera may not be anticomplementary to the serum complements of certain other species, and can perhaps be tested by the conglutinating complement absorption test.

Also it seems that the principles of the conglutination reaction afford a method of investigating the properties of the complements of different animal species, which up to now have scarcely been examined, simply because they do not exhibit haemolytic activity. We think therefore that further research into the mechanism of the reaction may well yield information of fundamental immunological interest.

Lastly, the confusion in the literature on this reaction demands that the whole subject be further investigated in the light of present knowledge in order to assess the true value of the conglutination phenomenon as a serological procedure.

For this reason we are publishing a review of the literature on the subject in the form of a statement of the findings and views of previous workers. Many of these views can be shown to be far from representing the true facts. We decided to deal with the subject in this manner because the literature is not only very widely scattered and uncorrelated, but appears mostly in foreign journals. Apart from the detailed discussion on this reaction and the bibliography compiled by Streng in 1929, no recent review has been published to our knowledge. We hope that this précis of the literature may be found helpful, in that it may form a starting-point for other workers wishing to contribute to the investigations on conglutination.

A technique for carrying out conglutinating complement absorption tests has been devised, and is described in detail in a second paper (Hole & Coombs, p. 490). The first disease to which this technique was applied by us was glanders. The investigation was carried out on experimentally infected ponies, and it may be stated that the conglutinating complement absorption test proved superior to the haemolytic complement fixatio test (p. 497).

It is our intention to investigate the possible application of this reaction to various diseases, and at the same time to work on the more academic and theoretical aspects which may lead to a fuller understanding of the nature of complement adsorption by an immune aggregate.

II. REVIEW OF THE LITERATURE

(*a*) *The development and acceptance of conglutination as a distinct phenomenon*

The phenomenon to which the name conglutination has been given was first observed by Ehrlich, although at the time he did not recognize it as a process distinct from that of immune haemolysis. It was Bordet & Gay (1906) who first described it as a separate and distinct phenomenon.

In 1902 Ehrlich & Sachs, while carrying out experiments on the nature of haemolysis of red blood cells by fresh serum, observed that fresh horse serum, together with heated bovine serum strongly haemolysed guinea-pig red blood cells. The fresh horse serum had no visible action on the cells or on cells which had previously been exposed to heated bovine serum and subsequently washed before adding the fresh horse serum. Also, the heated bovine serum after being absorbed with the red cells lost none of its capacity for bringing about haemolysis of guinea-pig cells, when active horse serum was also added. Ehrlich had previously stated that the immune body or 'interbody' consisted of two parts, the haptophore or cytophile group which combined with the red cell, and the complementophile group which combined with the complement. In his opinion the cytophile group of the immune body did not combine with, or act on the red cell, until the complement had united with the complementophile group. Here then to Ehrlich was an instance apparently proving his theory of immune haemolysis, namely, haemolysis caused by complement and immune sera. He assumed that in this case the immune body was in the heated bovine serum, and did not combine with the red cells before the affinity of its complementophile group for complement had been satisfied—in this case by the fresh horse serum. Ehrlich put the above experiment forward as his 'Experimentum crucis' which was intended to prove that an immune body possessed a complementophile group.

Bordet & Gay (1906) in a paper 'Sur les rélations des sénsibilisatrices avec l'alexine' showed that other factors were involved in this particular case of haemolysis of guinea-pig red cells. They showed that there existed an immune body for guinea-pig cells in the equine serum also. This immune body as that in bovine serum and all other immune bodies did combine with red cells in the absence of complement. The absence of haemolysis of the cells by the fresh horse serum was no proof that the cells were not in fact sensitized. The reason lay rather in the complement of the horse not being strongly haemolytic. They also showed that there existed a special substance in bovine serum, resistant to heating at 56° C. for half an hour, which had the property of being adsorbed by sensitized cells that had previously adsorbed a complement, not by itself haemolytic. This special substance had the effect of 'les agglutiner énergiquement, et de les rendre, sauf dans certaines conditions particulières, plus accessibles a l'hemolyse'.* This substance in bovine serum was not adsorbed by cells which were sensitized by an immune body alone, but acted only on the aggregates after complement had been adsorbed. That was the reason why the cells were not clumped and haemolysed unless the fresh horse serum and inactivated bovine serum were added together. So Bordet & Gay refuted the only argument which seemed at first sight to favour Ehrlich's hypothesis of haemolysis and claimed support with this new reaction, so far unnamed, for their hypothesis that the immune reactions could be explained in terms of biological adsorption.

Sachs & Bauer (1907) challenged these findings of Bordet & Gay and added their support for the Ehrlich school of thought. Bordet & Streng (1909) replied to this in a paper entitled 'Les phénomenes d'absorption et la conglutinine du serum de bœuf'. They reviewed their previous statements and answered the objections of Sachs & Bauer. They emphasized the clumping of the cells which occurred previous to haemolysis in the above system—a fact which it seems hardly attracted the attention of Ehrlich and his school. To this special substance in bovine serum they gave the name conglutinin, and the name conglutination to the phenomenon. They showed that to produce conglutination of blood cells an immune body, a complement and conglutinin were all required and that other complements besides that of the horse could be used. Conglutinin could be absorbed from bovine serum by sensitized cells which had previously also been treated with horse complement, although it was difficult to remove it completely. Gengou (1909) showed that the factor bringing about this clumping effect and the 'auxilytic factor' in bovine serum were different substances. Conglutinin causing the clumping or conglutination was a euglobulin while the 'auxilytic factor' was to be found in the pseudoglobulin fraction.

Conglutination seemed a very descriptive name for this phenomenon—suggesting the 'act of gluing together' although, as pointed out by Gay & Lucas (1909) the term had previously been applied to describe the agglutination of red blood cells by ricin.

Streng (1909*b*) demonstrated the phenomenon of conglutination using bacteria instead of red blood cells and stated that the apparent strong agglutination of certain bacteria, such as diphtheria bacilli, tubercle bacilli, pertussis bacilli and coliform bacilli,

* The primary agglomerating action was in fact due to conglutinin, while the later haemolytic action was due to an auxilysin—two distinct substances.

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by fresh bovine serum was really a process of conglutination and not of agglutination. Inactivated bovine serum no longer 'agglutinated' the bacilli, but the 'agglutination' or rather conglutination could again be demonstrated if to the inactivated bovine serum was added a fresh complement, which in itself did not agglutinate the bacteria. In fresh bovine sera there were to be found normal antibodies against most organisms as well as a complement and conglutinin—all the reagents that were necessary to produce conglutination. On heat inactivation of the bovine serum the complement was destroyed with the result that conglutination could not take place unless another source of complement was added. This powerful 'agglutinating' action of fresh bovine serum had often been recorded earlier. Braun (1909), for instance, had noted its strong agglutinating action on cholera vibrios. Romberg (1902) and Thellung (1902) had recorded similar observations in the case of tubercle bacilli. Most writers had explained these observations on the basis of the thermolability of normal agglutinins. On the strength of these findings Streng stated that conglutination was specific in that it required the intermediary action of an immune body, and suggested its use as a diagnostic serological procedure because it could detect sensitization by an immune body in itself too weak to cause agglutination.

Independently of the work of Bordet & Streng, Muir & Browning (1906) described the 'agglutinating' action of fresh ox serum on ox cells sensitized with an immune body from the rabbit. These authors explained their observations by assuming that fresh bovine serum contained an 'agglutinating complement'. However, it can be seen that the correct explanation is that the strong clumping of the cells was a manifestation of conglutination, due to the presence of complement and conglutinin in fresh ox serum.

Bail (1909) was another opponent of the Bordet school and did not recognize conglutination as a separate phenomenon to that of agglutination. Bail had always considered agglutination itself to be a very complex reaction, requiring a thermostable and thermolabile factor in serum for its manifestation. However, in his opinion, much less thermolabile factor (complement) was required to bring about agglutination of sensitized blood cells than was needed to cause their haemolysis. He considered that after heating a serum at 56° C. for half an hour there was still sufficient of this labile factor left to bring about agglutination, although not enough to bring about haemolysis. In these examples of so-called conglutination he thought he saw support for his complex hypothesis of agglutination. He disregarded conglutinin altogether, not differentiating it from an agglutinin, but stressed the importance of the complement. He did not see the need to surmise the existence of a substance, conglutinin.* His criticisms of the work and deductions of Bordet & Streng were set out in a paper in 1909.†

Once again Streng (1909*c*) replied, this time to the criticisms of Bail, in an article entitled 'Agglutinin oder Konglutinin?' in which he stressed the differences between conglutinin and agglutinins. Agglutinins could be absorbed by the native homologous cells while conglutinin could not. Also the one could be separated from the other by dialysing bovine serum against distilled water. Conglutinin was precipitated with the euglobulins, while the agglutinins remained in the supernatant fluid as pseudoglobulins. That conglutination was due to a summation of action of agglutinins could be disproved by absorbing out any agglutinins in the bovine serum before the test.

The controversy still went on. Spät's investigations (1910) on this phenomenon of 'agglutination' with normal bovine serum led him to conclude that the flocculation which occurred on the addition of normal bovine serum to bacterial suspensions could be comprehended only as an agglutination phenomenon and did not require a different reaction process, i.e. conglutination to explain it. He merely stated that the experiments of Streng brought new proof of the complexity of agglutinins. Spät, in his experiments, was not able to differentiate conglutination from agglutination in the following system—suspension of typhoid bacilli or cholera vibrios, immune sheep serum, guinea-pig complement and inactivated bovine serum. He too considered that with tubercle and diphtheria bacilli, the much stronger flocculation produced by fresh rather than by inactivated bovine serum gave strong support to Bail's theory of agglutination and bacteriolysis. However, these experiments considered as support for Bail's theory were carried out with bovine serum, the only serum used commonly in the laboratory which contains conglutinin in demonstrable amount.

The reply to Spät's paper this time came from Barikine (1910), a disciple of the Bordet school. He had no difficulty in demonstrating conglutination of cholera vibrios and differentiating the reaction from agglutination. In these experiments he used an immune body from a rabbit, while Spät used an immune body from a sheep. Barikine also showed that complement and consequently conglutinin also were adsorbed on to specific precipitates, even if these were previously invisible to the naked eye, and

* It can be shown that in the absence of conglutinin the presence of complement under certain conditions tends to inhibit rather than aid agglutination.

† One of the main causes of confusion in these early controversies on the conglutination phenomenon is the fact that if the sera are used too concentrated the immune body may be sufficiently strong to cause agglutination by itself.

gave a reaction or conglutination greatly enhancing the specific flocculation. He concluded that the conglutination of specific precipitates would over and above its theoretical interest find a place as a serological diagnostic procedure owing to the fact that conglutinin with the aid of a complement was capable of revealing a specific reaction otherwise invisible to the naked eye.

The controversy as to the identity of this reaction virtually came to an end when Streng in 1910, making use of the fact that complement was an essential reagent in the mechanism of the reaction, developed a complement absorption test in an analogous manner to the usual haemolytic complement fixation test. The demonstration of such a modification of the conglutination phenomenon was clear proof of the difference between conglutination and agglutination.

(*b*) *The application of the reaction as a diagnostic procedure*

(1) *The direct conglutination reaction*

As mentioned earlier Streng (1909*b*), using typhoid bacilli, was the first to demonstrate conglutination of bacteria. He stated that the reaction was specific, in that it required the intermediary action of an immune body, and suggested its use as a diagnostic serological procedure, because it would detect sensitization by an immune body in itself too weak to cause agglutination. The test which was developed we have called the *Direct Conglutination Reaction* and it consists of adding a complement and a source of conglutinin, usually in the form of inactivated bovine serum, to an antigen antibody immune system, and by means of which the antibody may be demonstrated to a much higher titre than in the absence of complement and conglutinin.

The reaction was first put to practical application by Cohen in 1909; shortly afterwards by Gay & Lucas in 1909; Lucas, Fitzgerald & Schorer in 1910, and by Swift & Thro and Sauli in 1911. Cohen was able to distinguish more clearly the Pfeiffer-like bacillus (considered to be the aetiological agent of certain forms of cerebro-spinal-meningitis) from the ordinary Pfeiffer bacillus, by applying the conglutination reaction than by agglutination or complement fixation tests. Gay & Lucas and Lucas, Fitzgerald & Schorer concluded that the conglutination reaction would possibly prove to be better than the agglutination test as a diagnostic procedure in cases of infantile dysentery. They stated that by using the conglutination procedure antibodies could be detected earlier in the course of the disease. Swift & Thro were not able to find any advantage of the direct conglutination reaction over the usual agglutination test in differentiating the various strains of streptococci obtained from different clinical conditions. Sauli, however, successfully made use of the conglutination test, in the same way as did Barikine, to enhance the serological precipitation of plant proteins by immune sera. He could so increase the specific precipitation as to demonstrate a heavy flocculation even when by the usual precipitation test no precipitate was visible to the naked eye. In this connexion the work of Gohlke (1913), Mez (1922), Mez & Ziegenspeck (1925) and Preuss should also be noted.

Murto (1914), using a direct bacterial conglutination test, stated that for diagnosing typhoid and paratyphoid infections in human beings the conglutination reaction was a better diagnostic aid than was the agglutination test. A similar conclusion was also reached by Ryti in 1925. She found, however, that many human sera contained a normal antibody to these organisms and so an arbitrary titre had to be taken as an indication of a diagnostic reaction.

In 1945 one of us (R.R.A.C.) attempted to apply this phenomenon in the hope that it would detect sensitization of human red cells with the 'incomplete' Rh antibody. These experiments were unsuccessful for certain technical reasons and the investigations were ended because another serological procedure was found suitable. A few months later Wiener (1945) published a paper entitled 'Conglutination test for Rh sensitization'. However we cannot accept the procedure adopted by Wiener as an example of the conglutination phenomenon (Coombs, Mourant & Race, 1945).

(2) *The conglutinating complement absorption test*

In 1910 Streng, realizing that complement was required for conglutination, developed a complement absorption test in a similar manner to the Bordet-Gengou haemolytic complement fixation test. These two tests are quite analogous except that in the conglutination reaction, complements of such animals as the horse, cat and pig, which are not haemolytic under normal conditions, may be used, and the indicator system is not a haemolytic system, consisting of sheep cells and haemolysin, but a conglutinating system, consisting of sheep cells and inactivated bovine serum.

Streng (1910, 1911) first applied this conglutinating complement absorption test to the diagnosis of syphilis and compared it with the Wassermann reaction. He found good agreement between the two and advised the carrying out of both tests as a diagnostic routine in order to reduce the number of uncertain results obtained when only one method was used. Jacobaeus (1911), basing his observations on a smaller number of examinations, concluded that the two methods gave a 90% agreement, but also stated (probably due to the technique used in those early days) that there was less difference between a positive and negative result with the conglutination reaction than was obtained with the Wassermann

reaction. In most of these investigations the complement used was that of the horse. Other workers, who investigated the relative advantages of this reaction as compared with the Wassermann reaction for the diagnosis of syphilis, include Karvonen (1911), Siebert & Mironescu (1911), Dallafavera (1912), Hecht (1912), Siebert (1912), Bernhardt (1912), Wassermann (1912), Leschly & Boas (1914), Veress & Szabó (1913) and Pfeiler (1917).

Perhaps it may be said that the reaction was found most successful in the diagnosis of glanders in horses, asses and mules. The workers who carried out investigations on its use in this condition include Pfeiler (1914), Pfeiler & Weber (1912*a*, *b*, *c*, 1913, 1914, 1915), Andersen (1913), Poksischewsky (1913), Michin (1914), Schütz & Waldmann (1914), Stranigg (1913), Waldmann (1914, 1917), Kranich (1915), Schnürer (1915), Schutz (1915), Muller (1916), Biermann & Zschiesche (1917), Biermann (1917), Danek (1917), Graub (1917), Pohle (1917), Schoening (1917), Poppe (1919), Fitch (1916), Breyer (1923), and Poppe (1922). Gildermeister & Jahn (1915) investigated the use of this reaction inthe diagnosis of glanders in man.

Luger (1912) used the reaction as a complement absorption test in experimental and clinical cases of typhoid, paratyphoid, dysentery and cholera. In a case of typhoid he obtained a positive reaction, as shown by complete inhibition of conglutination, while by the usual haemolytic complement fixation test he could only demonstrate a trace of inhibition.

Wehrbein (1915) investigated the use of this method in the diagnosis of dourine, a trypanosomiasis of the horse. As antigen he used either a suspension of the trypanosomes or spleen emulsion of infected rats. He found it especially suitable for investigating donkey's sera, which he stated were not satisfactory for use with the usual haemolytic complement fixation test on account of their anti-complementary action. He concluded that the conglutinating complement absorption method could be employed for the diagnosis of dourine, but was more sensitive to faulty technique and hence more difficult to use than the haemolytic test. In 1917 he reinvestigated its use in the diagnosis of this disease, and came to the same conclusions. Aehle (1923) also investigated its use in the diagnosis of dourine and found it a sensitive diagnostic test.

Reeser (1914) found the reaction unsuitable for the diagnosis of contagious abortion in cattle. Brocq-Rousseau, Urbain & Cauchemez (1925), from a comparatively small number of investigations, concluded that for the diagnosis of glanders it was as good as the usual haemolytic complement fixation test. For human and bovine tuberculosis they found it inferior. They also carried out a few tests with anti-anthrax sera, anti-streptococcal sera and syphilitic sera.

In more recent times Polyakov & Katyaeva (1933) compared the conglutinating and haemolytic complement absorption reactions in the diagnosis of contagious bovine pleuro-pneumonia.* The sera of 3281 bovines were examined. The conglutination test seemed the more reliable method. Again, in 1943, Solovieff investigated the use of the conglutination reaction in foot-and-mouth disease. The sera of 1329 different animals, bovines, pigs, guinea-pigs, horses, rabbits and sheep, some normal, some hyperimmune to the virus and some convalescent, were examined by the conglutination test for a reaction with the homologous virus antigen. Of these sera 390 were also examined by the usual haemolytic complement fixation test. The results seemed very satisfactory; there were less doubtful reactions than were obtained when using the haemolytic test. The serological application of the conglutination reaction seemed a reliable method for establishing the biological immune state of an animal to a particular virus of foot-and-mouth disease.

In these investigations heat inactivated bovine serum served as the source of conglutinin although different complements were used by the various workers. As described, most of the reports on the application of this reaction were favourable, especially so in the diagnosis of glanders. However, many workers considered that this reaction had no advantage over the ordinary haemolytic complement fixation test. This fact and the greater complexity of the test were probably the reasons why it gradually fell into disuse, with the result that to-day the reaction is no more than a name to many serologists.

In 1941 one of us (N.H.H.) was directed to carry out investigations into the diagnosis of glanders. The agglutination, haemolytic complement fixation and conglutinating complement absorption tests were compared as diagnostic procedures in experimentally infected horses over a period of some 10 months. The results obtained in these experiments, which are the subject of a paper in this series, showed that by using the conglutination reaction antibodies could be detected to a higher titre and over a longer period of time than by using the haemolytic tests. It was the promising results obtained in these experiments which stimulated us to continue investigations into the more theoretical aspects of the reaction and to its further practical application in other conditions.

(*c*) *Investigations into the mechanism of the reaction*

The early papers of Bordet & Streng clearly defined the nature of the reaction in serological terms,

* The conglutinating complement absorption test was also used for the diagnosis of this disease by Walker in 1923 (see also Heslop, 1921).

the mechanism being explained in terms of biological adsorption. However, the actual biochemical or physico-chemical basis of the mechanism still remains obscure although perhaps no more so than the actual mechanism of immune haemolysis.

Since these early papers by Bordet & Streng many workers have attempted explanations of the mechanism of the reaction in biochemical terms but in our opinion none of these can be considered satisfactory. Maltaner & Johnston (1921*a*, *b*) in two papers entitled 'Observations on the agglutinative and haemolytic action of calf serum on sheep cells' and 'Observations upon the conglutination phenomenon' identified conglutinin with the fibrinogen remaining in the serum after the clotting of bovine blood. In their opinion conglutination was due to this residual fibrinogen, which acted with certain components in fresh serum to form a fibrin clot around the cells and in this way to clump them non-specifically.

In 1925 Tagawa put forward an hypothesis on the mechanism of conglutination but this cannot be held of any account.

In America, Eagle (1930) concluded that the phenomenon was simply a specific instance of the general property of complement mid-piece, involving sensitized cells and the slight quantities of mid-piece present in heated sera which he maintained was conglutinin. He stated that the phenomenon was nothing more than a marked agglutination. He further concluded by stating that since the terms conglutinin and conglutination did not define a distinctive substance or reaction they should be dropped from immunological nomenclature.

Gyorffy (1932*a*, *b*, 1933, 1934), in Hungary, concluded from his experiments on the conglutination reaction that the mechanism was as follows. In the course of normal or immune haemolysis or bacteriolysis, substances of a kinase-like nature were set free, and acted on the residual fibrinogen (which he too associated with conglutinin) in bovine serum, causing a coagulation involving the cellular elements which led to the flocculation or conglutination. In his opinion the phenomenon should be called 'Scheinagglutination' or 'pseudo-agglutination' instead of conglutination.

In more recent times Matuoka (1939) in Japan studied the conglutination reaction but added no new facts to the knowledge of its mechanism. However, in 1940, Kadaya, Matuoka & Kanayama reported on the mechanism of the 'binding' of conglutinin and the separate components of complement during the process of conglutination. They merely recorded their conclusions without publishing their experimental work. They stated that mid-piece and component 4 of complement first combined with the sensitized cells. These 'persensitized' cells were then conglutinated by conglutinin after it had previously combined with end-piece.

It was at this stage that we began our investigations on this problem which we hope to publish in due course.

(*d*) *Other literature having a direct bearing on the conglutination reaction*

In a paper in 1911 Dean discussed the factors involved in agglutination. He demonstrated how normal serum globulin, especially of the guinea-pig, but also of other animals, had the property of enhancing specific agglutination. Complement was not necessary for this enhancing effect. In the same paper Dean also briefly reviewed the phenomena of conglutination and co-agglutination. He clearly stated that for conglutination complement was required and thus inferred that the 'auxi-agglutinating' effect of heated guinea-pig serum globulin was distinct from conglutination. Gengou (1909), in describing a similar phenomenon to that of Dean, also stated that it could not be an example of conglutination since that word had been reserved for the 'agglutinating' phenomenon which required the action of complement (contrast the 'Conglutination reaction' of Wiener (1945)).

In the early papers on conglutination, the action of conglutinin was considered as twofold; first, to bring about the clumping of sensitized cells which had adsorbed complement, and secondly to aid their haemolysis. However, it could be shown that the clumping action was due to one substance, namely conglutinin, while the auxilytic effect was due to another substance which may be present in the pseudoglobulin fraction of the same serum (Gengou, 1909; see also Manwaring, 1906). Streng (1909*a*) demonstrated the presence of conglutinin in the serum of other ruminants besides the bovine. He was unable to show any conglutinin in the sera of cats, pigeons, dogs, poultry, rabbits, pigs or of man. However he concluded that guinea-pig serum contained a very weak conglutinin-like substance and that horse serum seemed to contain a slow working, but apparently strong, conglutinin (see also Wartiovaara, 1932). Kujumgiev (1943) examined the serum of 'Hausbuffels', buffaloes, which are used a great deal domestically in Bulgaria, and found the conglutinin content of their sera to be of the same order as in other bovines.

The variation in the conglutinin content of bovine serum in health and disease was investigated by von Jettmar (1923). He found that in the diseases studied, whether the animals had fever or not, there was a fall in the conglutinin content of the serum, which returned to normal on recovery.

Hall, in 1913, carried out some experiments in an attempt to solve if possible the chemical identity of conglutinin. He was able to show that it was not ether soluble and that it was precipitated in the euglobulin fraction of serum by ammonium sulphate.

Browning (1925) recorded some experiments on the absorbability of complement and conglutinin by Berkefeld filtration.

Investigations on factors influencing the reaction were carried out by Leschly in 1915. He experimented on the effect produced on the velocity of the reaction by increasing the various reagents used in the test. He also investigated whether both complement mid-piece and end-piece were necessary for the reaction, and the range of pH over which the complement was active. Similar work was carried out by von Jettmar in 1923. He investigated the use of certain complements in different systems and recommended the complement of dog serum as being the most favourable—more so than horse complement. He also investigated the use of red cells from various species of animals. Many of the cells were ill-suited for the test because certain sera contained strong haemagglutinins for them.

About this time in Finland, Streng and his colleagues reported some further work on the conglutination phenomenon. In a paper entitled 'Vergleichende Untersuchungen über die agglutination und die konglutination der Blutkorperchen und Bakterien in Neutralsalzlosungen', Streng & Ryti (1923) showed that by replacing the sodium chloride medium used in serological tests with an equi-molar solution of sodium citrate agglutination was unaffected or, if anything slightly enhanced, whilst conglutination was completely inhibited. The sodium citrate was shown to inhibit the adsorption of complement on to sensitized cells, and also inhibited conglutination by the action of conglutinin on sensitized cells which had previously adsorbed complement in a sodium chloride medium. The inhibitory action of other salts upon this phenomenon and that of immune haemolysis was further reported by Ryti in 1925.

We think the following three papers on 'anti-complements' should be recorded on account of their important bearing on the phenomenon of conglutination. In 1908 Streng used the conglutination phenomenon to demonstrate the existence of a so-called 'anti-complement' to equine complement, which neutralized the complement's action and inhibited the subsequent conglutination by the action of conglutinin. Moreschi (1911), in a paper entitled 'Untersuchungen über die Funktion des Pferdekomplements als Antigens', showed that blood cells sensitized with an amboceptor and treated with horse complement could be 'agglutinated' by a rabbit anti-horse serum, due, as Moreschi stated, to a specific affinity between the complement and the material present in the antiserum. It will be noted that Streng did not observe 'agglutination' by his antiserum (anti-complement) but an inhibition of the action of the subsequently added conglutinin. Horse complement was chosen for this work because it could be adsorbed on to sensitized red cells without haemolysing them. In the third paper Perussia (1911) was able to produce an anti-substance by injecting rabbits with sensitized cells, which had also been exposed to fresh horse serum, and subsequently washed. This 'anti-substance' could specifically agglutinate cells which had adsorbed horse complement. Perussia's object in this study had been to show the antigenic function of horse complement.

This work on 'anti-complements' was continued by Streng in 1930. In a paper entitled 'Immuno-konglutinin-Antikomplement', he showed by immunizing rabbits with bacteria or blood cells, which had been washed after specifically adsorbing complement, that the rabbits produced antisera which reacted with complement when it was specifically adsorbed on to heterologous sensitized bacteria or blood cells and which caused the flocculation of the cells. On the other hand the antisera had no observable action on native bacteria or blood cells. The immune body was in a sense an 'anti-complement'. An immune body to adsorbed guinea-pig or sheep complement would also react *in vitro* with adsorbed equine complement. These immune bodies were thus in a sense analogous to the conglutinin in bovine serum and Streng suggested the name 'immuno-conglutinins' for them. Preliminary results of the use of immuno-conglutinins in diagnostic conglutination tests were published by Streng & Wartiovaara in 1930. Their use seemed quite satisfactory in cases where normal bovine conglutinin had previously been used. Wartiovaara (1932) in Helsinki undertook an extensive study of the development of the conglutinating property in serum on immunization. He verified Streng's findings, producing powerful immuno-conglutinins in rabbits by intravenous or subcutaneous injections of bacteria treated with guinea-pig complement, although by the latter route the titre produced was lower. Intravenous immunization with untreated bacteria produced immuno-conglutinins as well as agglutinins, although none were produced by the subcutaneous route. Intravenous injections of both active and inactivated sera produced low titre immuno-conglutinins. The specific bacterial agglutinins and the conglutinating property of the sera increased and decreased broadly speaking simultaneously during the course of immunization.

Another interesting observation on this reaction was recorded as early as 1911 by Gengou, who carried out some experiments on the flocculating action of sera on suspensions of gum mastic and starch. Under certain conditions the mechanism by which this flocculation is brought about can be considered as an example of conglutination, being dependent in the case of gum mastic suspension on a preliminary non-specific adsorption of complement.

In concluding this section it should be mentioned that Bordet has again discussed this reaction in a chapter of the 1939 edition of his book *Traité de l'Immunité dans les Maladies Infectieuses*.

IV. SUMMARY

1. A brief introduction to the Conglutination Phenomenon and to the Conglutinating Complement Absorption Test is given.

2. The reasons are stated why we think that further investigations into this phenomenon are desirable.

3. An account is given of the publications on the mechanism of the reaction, the hypotheses regarding it and its applications since the original observations of Ehrlich & Sachs in 1902.

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