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Excerpt from Dr. O. C. Strong, Studien über das Verhalten des Rindenserums
gegenüber den Mischungen, Zeitschrift für Bakteriologie, Parasitenkunde
und Infektionskrankheiten, Vol. 30, 1907: 47-80

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FURTHER DIFFERENCES BETWEEN CONGLUTINATION AND AGGLUTINATION

In order to determine the differences between conglutinins and conglutination on the one hand and agglutinins and agglutination on the other hand, I have carried out the following completed studies:

a. Can the conglutinin be replaced by the agglutinin? The following study where an inactive bovine serum, deprived of its corresponding sensitizers by prior contact with typhus bacilli, was placed with a 24 hour old typhus bouillon culture.

9 tubes were set up as indicated on Page 61.

All tubes were placed in the thermostat at 37°C and agitated from time to time simultaneously and in equal amount.

Tubes 1 and 6 showed no variation; tubes 7, 8 and 9 showed an agglutination after 20 minutes which isn't stronger in the presence of alexin; tubes 2, 3 and 4 to which alexin was added showed an agglomeration which was significantly later and slower. In contrast tube 5 showed an agglomeration not at all comparable to the others in 10 minutes. The only difference between tube 2 and tube 5 was that here in tube 5, inactive bovine serum freed from eventual sensitizers and agglutinins was also used. Although the agglomerating substance of the bovine serum had by itself no evident effect, tube 6, it still acts so strongly under the given conditions, that is, in the presence of sensitizers and alexin that the bovine serum cannot be replaced by another distinctly agglutinating serum from rabbits not even by somewhat larger doses of this serum (Tube 3, 4).

b. The agglutinin of a serum are absorbed from the serum by contact

with microbes which were not pretreated. Can one also remove the conglutinin from the serum with such microbes, that is, with unsensitized and unalexinized microbes? In order to answer this question the following study was made:

I allowed the sediment from 20cc of typhus bouillon culture to act for 3 hours on 0.2cc bovine serum + 0.8cc physiological salt solution. The bovine serum which was centrifuged, generally gave neither by itself nor together with guinea pig alexin, any reaction to typhus bacilli. How is it that only one such bovine serum is exhausted and another bovine serum unexhausted against typhus bacilli in the presence of alexin and a typhus immune sensitizer from rabbit serum?

The following mixings were made:

Tube 1: 0.5cc typhus broth culture + 0.1cc guinea pig alexin + 0.1cc immune serum + 0.2cc unexhausted bovine serum + 0.8cc physiological salt solution.

Tube 2: 0.5cc typhus broth culture + 0.1cc guinea pig alexin + 0.1cc immune serum + 0.2cc exhausted bovine serum + 0.8cc physiological salt solution.

Tube 3: 0.5cc typhus broth cultures + 0.1cc guinea pig alexin + 0.1cc immune serum + 0.8 cc physiological salt solution.

After a 15 minute stay in the thermostat, Tubes 1 and 2 showed a typical, strong agglomeration, Tube 3 showed a much weaker reaction.

The study showed that the bovine serum did not lose its colloidal substance through contact with native microbes, on the contrary the bovine serum both when it is previously exhausted with typhus bacilli and also when it has not been in contact with these microbes shows an equally strong reaction as

long as only a sensitizer and alexin are present. The parallelism with the results obtained with blood corpuscles (Ehrlich and Sachs, Sachs and Bauer, Bordet and Gay, Gordet and Streng) is here complete. As Bordet and Gay, and Bordet and Streng have shown, in contrast, the sensitized and alexinized blood corpuscles remove the colloidal substances from the bovine serum. By analogy one can probably conclude from this that the sensitized and alexinized microbes did this also.

c. How are the agglutinin, and the conglutinin which acts only on sensitized and alexinized microbes, retained during dialysis?

One certainly knows that the usual agglutinin will not be precipitated in the case of dialysis. Still after a month long dialysis they remain for the most part, up to 90% in solution (Widal; Achard and Bensande; Winterberg; Pich; and others). How is it certain that the conglutinins work on the microbes? Can one separate the latter from the agglutinins during dialysis?

Inactive bovine serum was dialyzed for 24 hours with tap water, the precipitate separated by centrifuging and again dissolved in a physiological salt solution. The decanting was again reduced to the physiological concentration by means of a concentrated salt solution and both these solutions were replenished with physiological salt solution of the same volume. The fluid which contained substances, which were precipitated in the dialysis and which were redissolved in physiological salt solution, is noted by A, the decanting by B.

As mentioned, a true agglutinin exists in bovine serum not only against typhus but also against Coli bacteria, which one can thus detect since

inactivated bovine serum in 1/2 hour at 56°C, which this contains no more alexins, will agglomerate these microbes. Which of the two fluids, A and B, contains only the agglutinin against typhus- and coli-bacilli?

4 tubes were prepared in the following way:

1. 0.5cc typhus broth culture + 0.3 fluid A
2. 0.5cc typhus broth culture + 0.3 fluid B
3. 0.5cc coli broth culture + 0.3 fluid A
4. 0.5cc coli broth culture + 0.3 fluid B

All tubes were placed in the thermostat at 37°C. The result showed that fluid A agglutinated both typhus and coli bacilli while fluid B showed a clear reaction. The bovine serum agglutinin thus remains dissolved in the serum for the 24 hour dialysis.

We again prepared 4 tubes as above adding to the microbes corresponding immune sera + alexin. As an immune serum we used typhus and coli-immune serum heated at 56°C, as alexin we used guinea pig serum.

Tube 1 0.5cc typhus broth culture + 0.05 typhus immune + 0.1 alexin + 0.3 fluid A

Tube 2 0.5cc typhus broth culture + 0.05 typhus immune + 0.1 alexin + 0.3 fluid B

Tube 3 0.5cc coli broth culture + 0.05 coli immune + 0.1 alexin + 0.3 fluid A

Tube 4 0.5cc coli broth culture + 0.05 coli immune + 0.1 alexin + 0.3 fluid B

The tubes were placed in the thermostat at 37°C. Tubes 1 and 3 showed a very strong agglomeration, while no reaction was observed in Tubes 2 and 4.

We conclude that the undissolved part of the bovine serum, which contains no agglutinin gives the agglomeration in the presence of alexin. This crossed procedure of the two portions of the bovine serum prove in my opinion that the same agglutinin and that colloid substance which is active in the case of

microbes, the conglutinin, are completely different.

d. We know that the presence of alexin is a prerequisite for conglutination.

What effect has the presence of alexin on the agglutination?

Since I will soon publish my exhaustive studies on this question, the retardation of agglutination by the complement, will only repeat the following experiment which was conducted simultaneously with the one described above.

We prepared the following tubes:

- Tube 1 0.5 cc typhus bouillon culture + 0.05 cc typhus immune agglutinin from rabbit + 0.1 cc guinea pig alexin
- Tube 2 0.5 cc typhus bouillon culture + 0.05 cc typhus immune serum from rabbit + 0.1 cc guinea pig alexin
- Tube 3 0.5 cc coli bouillon culture + 0.05 cc coli immune agglutinin from rabbit + 0.1 cc guinea pig alexin
- Tube 4 0.5 cc coli bouillon culture + 0.05 cc coli immune agglutinin from rabbit + 0.1 cc inactive serums from the same guinea pig

The tubes were all placed in the thermostat at 37°C. Tubes 2 and 4 rapidly showed (in 1 hour) agglutination. Tubes 3 and 4 gave, if the used immune agglutinin was not especially strong, no reaction and in any case always a significantly weaker reaction than in Tubes 2 and 4.

The study thus showed that the presence of alexin; which for the origin of the special conglomeration which I found in the presence of microbes was a *conditio sine quo non*, hindered the appearance of agglutination.

Conglutination is thus not to be regarded as an expression for the increased sensitivity of microbes, by means of sensitizing and alexin absorption,

compared with the agglutination of bovine serum.

e. If the conglutination were to be interpreted only as a strengthened agglutination then one must also probably grant that in both phenomena, agglutination and conglutination must always go parallel, which means that where a stronger agglutination results there must also result a stronger conglutination. But this is not the case. Bordet and Streng make note of divergence in this regard in the case of blood corpuscles and I refer the reader to this work.

In the case of microbes we can also show such a divergence between agglutination and conglutination.

The inactive bovine serum agglutinizes in a dosage of 0.3cc clear typhus- and coli-bacilli, but causes no agglutination of tuberculosis and diphtheria bacilli. The bovine serum thus effects, through its agglutination, typhus and coli bacilli much more severely than it does diphtheria and tuberculosis bacilli. If one adds to this dose 0.3cc of inactive bovine serum; 0.1cc of guinea pig alexin, then one obtains by this mixture completely opposite results. In this case diphtheria and tuberculosis bacilli give a very strong reaction while the typhus bacilli which I used give a reaction which is only a little stronger or not stronger at all than that obtained with the inactivated bovine serum. The conglutination caused by the bovine serum is thus stronger for the diphtheria and tuberculosis bacilli than for the typhus bacilli. Still to be noted is the part that the guinea pig alexin which was used, exerted a visible effect on the typhus, diphtheria and tuberculosis bacilli.

This incongruity between agglutination and conglutination could not be explained if the conglutination were only a strengthening of the agglutination.

CONCLUSIONS

1. By heating for a duration of 1/2 hour at 56°C the bovine serum loses its agglomeration property against several microbes tuberculosis bacilli, diphtheria bacilli, pertussis bacilli; the agglomeration however appears again if one replaces the alexin, inactivated by the heating, by some other more agglutinating alexin containing serum from guinea pigs, rabbits, horses or men.

2. This property of agglomeration of the bovine serum also disappears if one destroys the alexin by means of distilled water or if one removed the alexin of the bovine serum by prior contact with sensitive blood corpuscles, goat blood corpuscles, but it is formed again (i.e., the property of agglomeration) by addition of fresh alexin.

3. This agglomeration did not appear in spite of the presence of some alexin when the bovine serum due to prior contact with corresponding microbes has become deprived of its effective sensitizer, (diphtheria bacilli, tuberculosis bacilli of the bovine type, pertussis microbes, typhus bacilli) but arises even under these conditions if the removed bovine sensitizers are replaced by corresponding immunity sensitizers (pertussis, typhus, etc.). In some combinations the removed bovine sensitizers are replaced by normal sensitizers which exist in the serum which is used as the alexin source (B. coli, B. tuberculosis human type).

4. This agglomeration of the microbes is not changed with agglutination. In order to distinguish between the latter and the usual agglutination I proposed for this special agglomeration the name conglutination, which Bordet and Streng have proposed for an agglomeration of blood corpuscles which is dependent on the presence of alexin and the sensitizer and which is due to the

inactive bovine serum and which was found analogous by Bordet and Gay.

5. The active substance, in the case of conglutination, of the bovine serum which I will call the conglutinin could be separated by dialysis from the agglutinin.

6. The conglutination is not to be so understood that the microbes through sensitizing and alexin assimilation had received an increased sensitiveness against the usual agglutinin. From microbes prepared in such a manner the agglutinin which was separated by dialysis from the conglutinin acted more weakly than that which was not pretreated, while the alexin compound is a preliminary condition for the action of the otherwise inactive conglutinin. If the alexin became bound then relatively strong agglutinins could still not replace small amounts of conglutinins.

7. The conglutination phenomena do not always run parallel to the agglutination phenomena: microbes which are strongly agglutinated by bovine serum are often weakly conglutinated and vice versa.

8. The conglutination for which the presence of sensitizers is a preliminary condition is specific as to the bacteriolysis and the agglutination and can be used as such to diagnostic purposes.

9. A serum diagnosis with the aid of conglutination is so made that the serum which is to be studied is inactivated at 56°C and the microbes are mixed. For this mixing an optional amount of alexin and inactive bovine serum were added. If the sensitizer, for the serum to be studied, against the microbes which are used, then a conglutination usually does not occur. In the case of some special saprophytic microbes sometimes the serum, except when used as a source of alexin, and the normal sensitizers which exist in inactive bovine

serum are eliminated by prior contact with corresponding microbes. Also, instead of a bovine serum so pretreated, a precipitate of bovine serum, deprived by dialysis of its agglutinins and sensitizers, which is dissolved in a physiological salt solution can be used.

10. A titration of different microbes of sensitizing amounts of normal and immune serums is in this way often possible in vitro due to falling dosages.

11. Conglutination often appears to be a more sensitive reaction than agglutination. Thus for example a conglutination still results in a study using 0.001 ccm of immune serum while for the same immune serum only at 0.005 ccm did an agglutination appear.

12. For the start of a conglutination the culture medium in which the microbes are reared appeared to be of importance (pertussis).

13. The pathogenity of the microbes appears to play a role in conglutination. The saprophytic microbes give a reaction which is identical in the presence of normal sensitizers, which appears pathogenically and virulently to demand the presence of immune sensitizers.

14. All of the microbes studied by us (B. tuberculosis, B. diphtheria and pseudodiphtheria, B. typhi, B. coli, V. cholerae asiaticae, Staphylococcus pyogenes aureus) give a conglutination in the presence of the three compounds, alexin, sensitizer, and conglutinin.

15. The suppression phenomena which appear in the case of agglutination do not appear to play so large a role in the case of conglutination as in agglutination.

16. The conglutination reaction can also be initiated with microbes which were killed by boiling or by formalin.