ON THE ACTION OF COMPLEMENT AS AGGLUTININ.

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In the course of our experiments on haemolytic sera we have met with the following phenomenon which appears of some interest. It consists in the agglutination of the corpuscles of an animal by its own complement through the medium of the corresponding immune-body derived from another animal, and was observed first in the case of ox's corpuscles, the immune-body used being obtained from the rabbit. The fundamental fact is that if a certain amount of immune-body and ox's complement be added to ox's corpuscles, scarcely any lysis of the corpuscles occurs but they become agglutinated into large masses which cannot be dissociated by shaking. The immune-serum from the rabbit contains some agglutinin, but the degree of agglutination produced by this is quite trifling compared with that seen when complement also is added. There is thus no doubt that the agglutination phenomenon depends on the cooperation of two substances in a manner comparable to what obtains in lysis. The following are the chief facts regarding the conditions of occurrence of the agglutination and the nature of the agglutinating substance in the ox's serum.

Firstly as regards dosage, a certain amount both of complement and immune-body is necessary. Taking as the standard (one haemolytic dose) the amount of immune-body sufficient to produce complete lysis of 1 c.c. of 5 per cent. suspension of ox's corpuscles in ·8 per cent. sodium chloride solution along with guinea-pig's complement, we find that the maximum agglutination is obtained by 3—4 doses of immune-body and ·2—·3 c.c. of ox's serum (complement). The addition of larger amounts of immune-body or of complement has some effect in increasing the agglutination but only to a trifling extent. It occurred to us that

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the agglutination might be due to the imperfect lytic action of complement, resulting in the production of an adhesiveness of the corpuscles, but we have found that the agglutination by the complement also occurs when the lysis is complete. If we take some suspension of ox's corpuscles, add several doses (say eight) of immune-body and produce complete lysis by a single dose of guinea-pig's complement, we get a clear fluid, in which nothing can be seen by the naked eye, and in which on microscopic examination the stromata or shadows are seen to be uniformly distributed. If then to such a fluid we add ·2 c.c. of ox's serum and place the mixture in the incubator for a short time, flocculi appear which are found to be composed of agglutinated stromata. Another example may be given. We have stated[1] that it is not possible to produce complete lysis of ox's corpuscles by ox's complement acting along with the immune-body mentioned, and we have found this always to be the case when the immune-body and the complement are added at the same time. Since writing our paper, however, we have observed that if the immune-body is added some time—say an hour—previous to the addition of the complement, then as a rule complete lysis does occur on the addition of the latter, and in such a case there is marked agglutination of the stromata. From these facts it is evident that the agglutination does not depend upon an imperfect lysis.

Another point worthy of note is that the agglutination passes off after some time, usually in five or six hours, at the room temperature. When this occurs the agglutination can be restored on the addition of more complement, whilst additional immune-body is practically without effect. As a rule this second agglutination is not quite so marked as the first. The passing-off of the agglutination is seen both in the case of the unlysed corpuscles and also in the case of the stromata.

We have also enquired into the temperature at which the agglutination occurs. It is seen within a few minutes (with the doses mentioned above) at 37° C. and in a not much longer time at the room temperature. At 0° C. on the other hand, even after a period of two hours, there is practically no agglutination visible to the naked eye. It would appear from this that the agglutinating action of complement was absent at this temperature, but if we centrifugalise the treated corpuscles it is found that they adhere in masses so firmly that they cannot be separated by shaking. It is thus shown that there is some slight action at 0° C., evidenced by abnormal stickiness of the corpuscles but not by the

[1] Muir and Browning, *Proc. Roy. Soc. London*, 1904, vol. LXXIV. p. 298.

spontaneous clumping seen at higher temperatures. We therefore cannot say that the agglutinating substance is entirely without action at 0° C., though it is much less marked than at higher temperatures. We may mention another case, viz. guinea-pig's corpuscles, immune-body from the rabbit, and ox's complement, with which combination there is marked agglutination by complement at 0° C., though of course there is no lysis.

When the ox's serum is heated for an hour at 55° C. its agglutinating property, like its haemolytic, is lost, such a serum having no effect when added to ox's corpuscles treated with immune-body.

From this short statement it is seen that the agglutinating body, studied in the normal serum of the ox, resembles haemolytic complement both as regards (*a*) its comparative lability—it is rapidly destroyed at 55° C., and (*b*) its acting only in association with immune-body. These circumstances justify the application of "complement" to it. It is not quite clear however whether this agglutinating complement and the ordinary lytic complement are one and the same substance. A study of the temperatures at which the two effects—agglutination and lysis—occur, shows that a slight difference exists. This may be due to there being what we may call two complements, or it may simply be due to one substance exerting the agglutinating effect at the lower temperature. Further observations will be necessary on this point. It is also to be noted that the production of adhesiveness of the corpuscles may be manifested before agglutination in the ordinary sense appears.

The facts noted show that the phenomenon of agglutination usually produced by a single body (agglutinin) possessed of combining and agglutinating groups, can also result from the cooperation of two substances in a manner completely analogous to what is seen in bacteriolytic and haemolytic action. Whether this agglutination of an animal's corpuscles by its own complement may be brought about in conditions of disease by some substance acting like an immune-body remains to be seen. If it does, it is manifest that very grave effects will result.