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ON THE PROPERTIES OF ANTI-IMMUNE-BODIES AND COMPLEMENTOIDS[1].

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OF papers recently published which concern the properties of immune-sera and bear upon the general theory of immunity one of the most important is that of Bordet[2] on *antisensibilisatrices* (anti-immune-bodies, anti-amboceptors). This observer obtained an anti-immune-body by injecting the serum of a normal animal (rabbit) into an animal of another species (guinea-pig), and found that there was developed an anti-immune-body which had the property of neutralising the various immune-bodies which might be developed by active immunisation of the first animal. In this way a certain community as regards combining properties was demonstrated among the immune-bodies of a given species of animal. He studied the properties of the anti-immune-body, and in particular showed that it did not combine with the cytophile group of the immune-body, and therefore did not prevent the usual combination of the cell-receptor with the immune-body. This fact he held to be inconsistent with Ehrlich's views regarding the amboceptor constitution of immune-body. His observations on the neutralising effect of the anti-immune-body were carried out by means of haemolytic sera. Ehrlich and Sachs[3] confirmed the chief results obtained by Bordet, but so far from admitting the establishment of any objection to

[1] Towards the expenses of this research a grant was given by the Carnegie Trustees, for which we have pleasure in recording our indebtedness.

[2] Bordet. *Ann. de l'Inst. Pasteur*, 1904, Tome XVIII. p. 593.

[3] Ehrlich and Sachs. *Berlin. klin. Wochenschr.* 1905, pp. 557, 609.

the amboceptor theory, claimed that they supplied strong evidence in support of it. They maintained in fact that the anti-immune-body acted by combining with the complementophile group of the amboceptor, and thus prevented the union of complement, stating also that the results showed that the various immune-bodies from the same species had a similar complementophile apparatus. The theoretical bearings of the facts established will, however, be discussed later.

As the subject appeared to us to be of high importance we have repeated the various experiments, and have in addition studied the combining properties of anti-immune-bodies by quantitative methods. We have especially enquired whether the combination of anti-immune-body can thus be demonstrated even when its action in preventing lysis of red corpuscles is not apparent, and have found this to be the case. In this connection we have been forced to consider certain circumstances which influence the occurrence of lysis, and have carried out observations on the effect produced by altering the medium of suspension of the red corpuscles. The present paper thus consists of two main parts, viz. (1) a preliminary part, dealing with the dosage of complement in different media, and (2) a part dealing with the main subject of the investigation, as stated above.

The methods employed are those described in former papers and need not be repeated. The test quantity of corpuscles generally used was half the usual amount, viz. ·5 c.c. of a 5 per cent. suspension of washed corpuscles in ·85 per cent. salt solution. The anti-immune-body was obtained by injecting guinea-pigs with the serum of the normal rabbit, a guinea-pig of about 500 grams receiving two injections of from 4 to 6 c.c. on two occasions, with an interval of ten days between, and then being killed about ten days after the last injection. Several guinea-pigs were thus treated at the same time and the various sera were mixed together and heated at 55° C. to deprive them of complement. Such a serum of course contains an anti-complement to the rabbit's complement, but as the guinea-pig's complement is used in the experiments this element does not come into play. The immune-body in most cases is that obtained by injecting rabbits with ox's corpuscles freed from serum by washing in salt solution. In some experiments we also used an anti-immune-body to the guinea-pigs' immune-bodies, which was obtained by injecting a rabbit with guinea-pig's serum.

The abbreviations used are the following, C = complement; IB = immune-body; anti-IB = anti-immune-body; R = receptor; M.H.D. = mini-

mum haemolytic dose, of IB or C as the case may be. The denomination of a serum is conveniently made by putting the name of the animal supplying it first, and the name of the animal on whose corpuscles the serum acts second. Thus serum "rabbit *v.* ox" means a serum obtained from a rabbit treated with injections of ox's corpuscles and thus acting on these latter. It is convenient, as Bordet has done, to indicate that a serum has been deprived of the action of complement by heating, to place 55° C. after its name, *e.g.* guinea-pig serum 55° means the serum of a normal guinea-pig, heated for an hour at 55° C.

I. On the dosage of complement in different media and on complementoids.

Before recording our observations on the action of anti-immune-body we shall first refer to a phenomenon observed by Bordet. He found that the anti-immune-body might be able to protect corpuscles treated with immune-body against the action of complement when the corpuscles were suspended in guinea-pig's serum 55° C., whilst the protective action might fail when they were suspended in salt solution. In other words, the action of the complement on the corpuscles was greater in the latter medium. We have made corresponding observations and can fully confirm Bordet's results. In explanation of the phenomenon he considers that the salt solution is a less suitable medium for the corpuscles than the heated serum of the guinea-pig, thus the haemoglobin diffuses out more readily. There is thus only a relative neutralisation of the immune-body—"la sensibilisation simplement atténuée produit encore ses effets si les globules sont maintenus dans un milieu diminuant leur résistance." He further considers that the fact referred to is somewhat analogous to the observations of Roux and Vaillard that a mixture of tetanus toxin and antitoxin might be harmless for normal guinea-pigs, and still be dangerous for guinea-pigs previously debilitated by vaccination against the cholera vibrio. We have considered the subject of some importance and have carried out observations with the following results.

It is to be noted at the outset that the corpuscles used are those of the ox, the immune-body is obtained from the rabbit by injecting it with ox's corpuscles, and the complement is that of the guinea-pig. It seemed to us a somewhat curious circumstance that the heated serum of the guinea-pig should be a specially suitable medium for suspending the corpuscles of the ox, and have considered it desirable to test the

haemolytic dose of complement when the corpuscles are suspended in different media, especially in the heated sera of different animals. Such heated sera, though bereft of complement or rather of the toxic action of complement, contain complementoids according to Ehrlich's view; this matter will be referred to below. So far as we know no observations of this kind have been made.

It is scarcely necessary to state that all the experiments were carried out with the different media of suspension at the same time and for the same periods of time. The results may be given in tabular form, and the doses are most conveniently given in terms of the minimum haemolytic dose in salt solution.

Ox's corpuscles. Immune-body from Rabbit. Guinea-pig's complement.

Medium of suspension	Haemolytic doses of complement necessary for complete lysis
·85 per cent. sodium chloride solution	1
Ox's serum 55°	2
Guinea-pig's serum 55°	5·5
Guinea-pig's serum 55° and ·85 per cent. salt solution, equal parts	3+
Rabbit's serum 55°	2+

N.B. The relative dosage varies considerably with different samples of serum but the figures given may be taken as a fair average.

It appears clearly from this table that the dose of complement necessary for haemolysis varies greatly according to the medium in which the corpuscles are suspended, and we cannot offer in every case an explanation; no doubt factors of different kinds are concerned. We will confine our attention to the case of haemolysis of the ox's corpuscles suspended in the heated serum of the guinea-pig. It will be seen that the dose of complement is in this case about three times the dose when the corpuscles of the ox are suspended in their own serum. In other words, the guinea-pig's serum would seem to protect the ox's corpuscles against the toxic action (complement) better than the ox's serum, or to be a more suitable medium in Bordet's sense. On theoretical grounds it appears that some other explanation must be looked for. It will also be seen that the dose of complement necessary even when the corpuscles are suspended in a mixture of equal parts of salt solution and guinea-pig's serum 55° is greater than when the corpuscles are in their natural medium.

We have enquired into the method by which the guinea-pig's serum 55° retards lysis, that is demands a higher dose of complement.

Theoretically there are at least two possibilities, the one being that the heated serum in question interferes with the combination of complement, the other that it in some way retards the diffusion of haemoglobin. We have found that the former is the chief factor in bringing about the result. If the corpuscles be suspended in heated serum of the guinea-pig and 3 M.H.D. of complement be added (M.H.D. being the minimum dose in salt solution) only slight lysis occurs in the course of two hours at 37° C. Now if at the end of this time the surviving corpuscles be removed from the serum, then washed and suspended in salt solution and placed in the incubator for another period, the corpuscles do not undergo lysis. Consequently complement has not entered into combination with them. (It is to be noted that of course multiple doses of immune-body were present to begin with.)

We have also shown that in such a case as that mentioned there is still free complement in the medium of suspension. We take two series (A and B) of tubes each containing ·5 c.c. of suspension of red corpuscles along with five doses of IB; the medium in A being salt solution, in B guinea-pig's serum heated at 55° C. To the several tubes in the two series increasing doses of complement are added. After lysis is complete in the tubes of series A we test as to the comparative amount of free complement in the two series. The fluid from each tube of series A is added to another tube containing ·5 c.c. of guinea-pig's serum 55°, the fluid obtained by centrifugalisation from each tube of series B is added to a tube containing ·5 c.c. of salt solution (·85 per cent.). In this way any free complement obtained from the tubes of either series is now in a mixture of equal parts of heated serum and salt solution. To each tube we now add the corpuscles of ·5 c.c. of suspension treated with immune-body and the tubes are placed in the incubator. It is found that lysis takes place much more readily in the tubes containing the fluid from series B, *i.e.* there was much more free complement in this series. Such an experiment is complementary to and confirmatory of the result recorded above.

Having thus seen that the combination of complement with corpuscles treated with immune-body is *interfered with* when guinea-pigs' serum 55° is the medium of suspension we have to enquire how this is brought about.

Now it is to be noted that the guinea-pig's complement (normal serum) is much the most active of the complements used, and that this serum when heated interferes with haemolysis more than the others.

In a former paper[1] we have given reasons for believing that there are more complement molecules in the guinea-pig's serum than (for example) in the same quantity of rabbit's serum. We have also given methods for demonstrating by test-tube experiments the existence of complementoids in heated sera, these of course being derived from complements. Let us suppose that the haemolytic dose of guinea-pig's complement for the test 1 c.c. of suspension of corpuscles in salt solution is ·01 c.c. and that each molecule of complement gives rise to a molecule of complementoid. Then when the same amount of corpuscles is suspended in heated serum instead of salt solution and ·01 c.c. of fresh serum is added there will be a hundred molecules of complementoid for each complement-molecule—under this condition complete lysis does not occur. This is probably an exaggerated statement of the case, at least we were unable to show by combination tests that there were as many complementoid molecules as there were originally complement molecules. For the sake of illustration let us put the proportion at fifty complementoid molecules and one complement molecule. We have further shown that when complementoid is brought into contact with red corpuscles treated with immune-body only a small proportion of complementoid combines with them, so that when the corpuscles are afterwards washed and complement is added lysis is not interfered with. But it seems possible that when a large number of molecules with feebler affinity (complementoid) are actually present in the mixture the combination of those with stronger affinity (complement) may be interfered with. Furthermore when lysis occurs the combining affinity for complementoid molecules is much increased, complete lysis may thus be considerably interfered with by their presence. It therefore appeared reasonable to enquire what the effect would be if the complementoid molecules were *removed* from the serum. If our supposition is correct then lysis should occur much more readily, *i.e.* with a smaller dose of complement.

Now the complement action may be removed from a serum in two ways, viz. (*a*) by heating at 55° C., and (*b*) by bringing the serum into contact with substances for which the complement has a combining affinity. In the former case the complement is converted into inactive complementoid; in the latter, if the substance with which it is combined can be separated by centrifugalisation, the complement will be removed from the serum. In the former case if our theoretical considerations are correct the serum when used as a medium of suspension will interfere

[1] Muir and Browning. *Proc. Roy. Soc. London*, May 17, 1904.

with lysis; in the latter this interfering action should be absent. Acting on these ideas we have investigated how haemolysis will progress in a serum from which the complement has been *removed*. We can remove the complement from a fresh serum by bringing it into contact with some cells or bacteria treated with the corresponding immune-body and then after time has been allowed for combination remove the serum by centrifugalisation. In this way a serum practically free from complement as tested by haemolytic experiments is obtained. We have carried this out by various methods, the most satisfactory of which is the following. Washed corpuscles of the ox in ·85 per cent. salt solution are placed in a steriliser over night at 55° C., they are then centrifugalised and the brownish fluid is removed. The haemolytic receptors of such heated corpuscles are, however, not destroyed; they still have the power of combining with immune-body and thereafter of taking up complement. To a suspension of heated corpuscles a large amount of immune-body is added, and after time is allowed for combination of the latter the corpuscles are repeatedly washed in salt solution and centrifugalised; finally the fluid of the suspension is removed as completely as possible, so as to avoid dilution of the serum. To the corpuscles thus treated the fresh serum of the guinea-pig is added and the mixture is thoroughly shaken up and placed in the incubator at 37° C. for two hours; the tubes are then centrifugalised and the serum is pipetted off. If sufficient amounts of corpuscles and immune-body are used the serum is rendered practically devoid of haemolytic action; sometimes there remains a trace of complement which becomes evident when relatively large quantities are used, as is the case when it is the medium of suspension of corpuscles. To get it entirely free of haemolytic action we heat it for an hour at 55° C.; the small amount of complementoid which may thus remain does not interfere with the test. We shall speak of the serum thus freed of complement as "treated serum," while serum heated at 55° C. will be designated "serum 55°." In addition to the method described we have also used, to take up the complement from the serum, red corpuscles combined with immune-body and freed from fluid. In this case of course the corpuscles when added to the serum undergo lysis, but by adopting certain procedures the haemoglobin-stained serum can still be used as a medium of suspension. In other experiments we employed an emulsion of kidney cells along with the corresponding immune-body, the cells being afterwards removed by centrifugalisation. All the results obtained have been of the same nature; but as the first-mentioned

method is the most satisfactory we need give details only with regard to it.

In estimating the haemolytic dose of complement in the "treated serum" we used in each tube the corpuscles of ·5 c.c. suspension, and of course ·5 c.c. of serum was added after the salt solution had been removed by centrifugalisation.

Doses of Guinea-pig's Complement in different Media of Suspension for the Corpuscles of ·5 c.c. Suspension.

·85 per cent. sod. chloride	Serum 55°	Treated Serum
·005	·03 (6 D)	·01 (2 D)
·0125	·06 (5 D)	·02 (1·6 D)
·004	·1 (25 D)*	·015 (3·75 D)

* This was by far the largest dose observed in any of our experiments and must be regarded as exceptional.

It thus appears that the dose of complement is very much smaller when the medium of suspension is treated guinea-pig's serum than when it is guinea-pig's serum 55°. In other words, the serum 55° exerts a strong inhibitory influence on lysis, which is not the case when the complement is removed from the serum instead of being changed into complementoid. The dose in guinea-pig's "serum treated" is in fact little, if at all, greater than that in ox's serum 55°. The high dose of complement necessary when serum 55° is used as the medium of suspension was supposed by us to be due to complementoid, and this view is fully confirmed by the results of using as the medium of suspension the serum deprived of its complementoid. We are thus justified in concluding that the presence of a large amount of complementoid interferes with the action of complement and thus raises the haemolytic dose.

These results are of importance in connection with the question as to the existence of complementoids and their combining properties, and constitute an amplification and confirmation of what we have published in former papers. Gay[1] in a recent paper criticises the well-known experiment of Ehrlich and Sachs[2] in which the complementoid of dog's serum prevents the action of guinea-pig's complement on guinea-pig's corpuscles sensitised with the natural immune-body in the dog's serum,

[1] Gay. *Centralbl. f. Bakteriol. u. Parasitenk.* 1. Abt. Originale, Bd. xxxix. S. 172.

[2] Ehrlich and Sachs, *Berlin. klin. Wochenschr.* 1902, no. 21. In a recent paper, *Centralbl. f. Bakteriol.* 1. Abt. Originale, Bd. xl. S. 125, Sachs has replied to Gay's objection and confirmed his previous results.

and comes to the conclusion that the supposed complementoid is merely an attenuated complement—attenuated both as regards its combining affinity and its toxic action. We would point out, however, that in our former experiments, as in the present case, the serum heated at 55° C. is quite devoid of haemolytic action, in fact so far as can be seen from the haemolytic action the complement has entirely disappeared. Nevertheless in such a serum a substance (complementoid) is present which combines with the same molecules as complement, viz. with anti-complement and with the complex receptors + immune-body. We claim in fact that the existence of complementoids has been demonstrated by test-tube experiments, and that Ehrlich's views regarding these bodies have been completely confirmed. It would in fact be impossible to explain the high dose of complement necessary when the corpuscles are suspended in guinea-pig's serum 55° on the theory that complementoids are merely attenuated complements, *i.e.* attenuated in combining affinity and toxic action in equal degree.

II. The properties of anti-immune-bodies.

Demonstrations of the action of anti-immune-body.

As stated above the anti-immune-body is obtained by injecting the guinea-pig with the normal serum of the rabbit. We shall speak of such a serum obtained from a guinea-pig as anti-IB; it is of course deprived of complement by heating at 55° C.

The fundamental result obtained by Bordet showing that the anti-immune-body unites with red corpuscles treated with immune-body and protects them against the action of complement is readily demonstrated. The following may be taken as an example.

The experiment may be represented thus:

A.	RCs + 3 IB + ·3 c.c. anti-IB	*W* + C	no lysis*
B.	RCs + 3 IB + ·3 c.c. guinea-pig's serum 55°	+ C	lysis*

The vertical interrupted line represents a period of incubation at 37° C., for an hour unless the time is stated.

W signifies that the corpuscles are washed and centrifugalised after incubation.

* The corpuscles are suspended in guinea-pig's serum 55° C.

Two series of tubes (A and B) each containing ·5 c.c. of suspension of red corpuscles are prepared, and to each three haemolytic doses of immune-body are added. After an hour to allow for combination the

corpuscles are centrifugalised and washed with salt solution (all the immune-body present is therefore in combination with the red corpuscles). To each of the tubes in one series (A) we add ·3 c.c. of anti-immune-body; to the tubes in series B ·3 c.c. of normal guinea-pig's serum heated at 55° C. as a control. The tubes are placed in the incubator at 37° C. and after an hour they are centrifugalised and the contents washed with salt solution. They are again centrifugalised and the salt solution is pipetted off. To each tube is added ·5 c.c. of guinea-pig's serum heated at 55° C. We have thus two series of tubes, in one of which the corpuscles are combined with IB and anti-IB, in the other only with IB. We then test the effects of complement by adding varying amounts to the several tubes. It is found that in series A ·1 c.c. of complement produces practically no lysis, whilst in series B the same amount produces complete lysis. In series A even ·2 c.c. of complement produces only slight lysis.

On the mode of action of anti-immune-body.

Does the anti-immune-body act by *preventing the union of complement* with the red corpuscles treated with immune-body or by in some way *inhibiting the toxic action* of complement? The former will be shown to be the case, if we separate the fluid from a tube in which lysis has been prevented by anti-immune-body and find that it contains uncombined complement, the test being made in the usual way by adding red corpuscles treated with immune-body and observing whether lysis results. Experiments carried out on these lines show clearly that in every case where the anti-immune-body prevents lysis, complement has been kept out of combination. This is in confirmation of the result arrived at by Bordet. In our initial experiments the red corpuscles were suspended in guinea-pig's serum 55°, in accordance with Bordet's method, but we afterwards used salt solution as the medium of suspension and investigated the action of anti-immune-body in general, even where lysis is not prevented. The details of method are of similar nature in the two cases and are given below.

Bordet found that the action of anti-immune-body could be demonstrated when the corpuscles are suspended in guinea-pig's serum 55° C., whereas this result was usually not obtained when they were suspended in salt solution. *A priori* it is unlikely that the action of anti-immune-body is not identical in the two conditions, and we have investigated whether this is the case. We find that in salt solution the

effect of anti-IB can be demonstrated in two ways, (*a*) by its delaying lysis, and (*b*) by its keeping out complement. We proceed as before by adding immune-body and anti-immune-body to the one series of tubes containing ·5 c.c. suspension of red corpuscles, and to the other series immune-body and guinea-pig's serum heated at 55° C. After the corpuscles have been washed, complement is added in varying amounts.

A. Red corpuscles + 5 doses of IB + ·25 c.c. anti-IB.
Amounts of guinea-pig's C ·01, ·015, ·02, ·025, ·03, ·04, ·05, ·06 c.c.
Dose of C = ·005 c.c.
Amount of C taken up in 1½ hours at 37° C. = ·012 c.c.

B. Red corpuscles + 5 doses of IB + ·25 c.c. guinea-pig's serum 55° C.
Amounts of C added as in A.
Amount of C taken up = ·023 c.c.

The difference in the amount of complement taken up in the two series is thus very striking, being ·011 c.c. or more than two haemolytic doses of C, this amount being kept out by the action of anti-IB.

The action of the anti-IB was also seen in the rapidity of the initial lysis in the two series. After twenty-five minutes, lysis in series A was just complete in the tube containing ·04 c.c. of complement, whilst at the same time in series B it was complete in the tube containing ·015 c.c. of complement; at the end of forty minutes, however, lysis was complete in all the tubes of both series. The action of anti-IB can thus be demonstrated quite clearly by the rate of occurrence of lysis when the corpuscles are suspended in salt solution, even though lysis is not prevented. The difference as regards lysis in the two media is due to the fact that a much larger dose of complement is necessary when the corpuscles are suspended in guinea-pig's serum 55°; that is, complement acts more feebly, and any further diminution in its action is much more apparent. We therefore conclude that whether lysis is prevented or not, the anti-immune-body in every case keeps a certain amount of complement out of combination.

Action of anti-immune-body on multiple immune-bodies developed by active immunisation.

Bordet found that anti-immune-body acted on all the immune-bodies supplied by the animal whose serum was injected in order to produce the anti-immune-body. In addition to the immune-body for ox's corpuscles we have also investigated by quantitative methods the action

of anti-immune-body in the case of the immune-body for guinea-pig's corpuscles (obtained by injecting a rabbit with these corpuscles).

Example. Dose of IB for guinea-pig's corpuscles = ·006 c.c.[1]

Two series of tubes, A and B, containing ·5 c.c. suspension of red corpuscles and four doses of immune-body, are prepared as before, those in A also receiving ·2 c.c. anti-IB—the details need not be repeated. In series A the amount of guinea-pig's complement taken up was ·015 c.c., in series B ·045 c.c. Therefore the anti-immune-body (·2 c.c.) kept out of combination ·03 c.c. of C. (The test for uncombined complement was ox's corpuscles treated with immune-body.)

It is interesting to note that in a similar experiment made at the same time with ox's corpuscles treated with four doses of immune-body the amount of complement kept out by ·2 c.c. of anti-immune-body was the same as in the case of guinea-pig's corpuscles, *i.e.* ·03 c.c. of complement, as tested with corpuscles treated with immune-body. In other words, apparently the same amount of anti-immune-body had been taken up by the two kinds of corpuscles treated with their corresponding immune-body.

Action of anti-immune-body on natural immune-bodies.

We have investigated this in the case of the anti-immune-body obtained by injecting the rabbit with guinea-pig's serum—the anti-immune-body thus neutralising the immune-bodies derived from the guinea-pig. The normal serum of the guinea-pig has a varying degree of haemolytic action on both rabbit's and ox's corpuscles, and this, as was shown by Ehrlich, is due to the combined action of natural immune-bodies with complement. By placing the corpuscles (rabbit's or ox's) in contact with the guinea-pig's serum at 0° C. a certain proportion of immune-body enters into combination, as is shown by the fact that when the corpuscles are washed in salt solution and complement is added a certain amount of lysis takes place. The method of testing the action of anti-immune-body is thus the following. The corpuscles are placed in the serum for an hour at 0° C. and are then washed free of serum. They are then divided into two sets, to one of which is added ·2 c.c. of anti-immune-body, and to the other, as a control, ·2 c.c. of rabbit's serum 55°. After an hour at 37° C. the corpuscles are washed and the same amount of complement is added to each. It is found that the resulting lysis is less in the case of the corpuscles treated with anti-

[1] *i.e.* along with rabbit's complement. On variations in dosage *vide* Muir and Browning, *Proc. Roy. Soc. London*, 1904, vol. LXXIV. p. 298.

immune-body. The difference is marked in the case of the ox's corpuscles; slight, though distinct, in the case of the rabbit's corpuscles. Anti-immune-body thus apparently acts in the same way on natural immune-bodies as on those produced by artificial immunisation. This is additional evidence, if such were needed, that the immune-body naturally present has the same constitution as that developed by active immunisation.

(*Note.* The "complement" used in these experiments was guinea-pig's serum which can be practically freed of the natural immune-body for ox's corpuscles by contact with the corpuscles at 0° C. In the case of the immune-body for rabbit's corpuscles, however, so complete a result is not obtained, though a certain amount of immune-body is taken up. Thus the serum cannot be completely freed of its natural haemolytic action. As there is, however, much more complement relatively than natural immune-body the action of anti-immune-body can quite well be demonstrated.)

Relation of anti-immune-body to the combination of immune-body with receptors.

We have seen that the anti-immune body acts by preventing the combination of complement with R + IB molecules. We have, however, considered it advisable to investigate by quantitative methods whether it has any action in preventing the union of immune-body with the receptors of the red corpuscles. The principle is to test whether the same amount of immune-body is taken up by the red corpuscles in the presence of anti-immune-body as when it is absent. This is best done by observing the saturation point of the red corpuscles with immune-body in the two conditions; that is, to find how many doses of immune-body must be added before one dose remains free.

Two series (A and B) of seven tubes, each containing ·5 c.c. of salt solution, are taken:

(1) To each tube in A 4 doses of IB and ·2 c.c. anti-IB,
„ „ B 4 doses of IB and ·2 c.c. guinea-pig's serum 55° (as a control).

They are placed in the incubator for an hour at 37° C.

(2) ·5 c.c. of suspension of red corpuscles is added to each, and the tubes are again incubated for an hour.

The contents of each tube are then washed and centrifugalised. The corpuscles in all the tubes have thus been treated with the same amount

of IB, those in series A being treated with anti-IB also. We have thus to test how much IB will be taken up in the two series.

(3) To the several tubes in the two series 1, 2, 3, 4, 5 etc. doses of IB are added.

(4) After an hour the tubes are centrifugalised and the separated fluid from each is added to red corpuscles along with a sufficient amount of complement, in order to show the amount of free IB in each.

The result is that lysis is the same in both series, being first complete in the fifth tube in each. As originally four doses of IB were added and then five to this tube, this means that whether anti-IB is present or not the same amount of IB is taken up—when nine doses of IB are added, one dose remains free.

We therefore conclude that the anti-immune-body in question has no effect in interfering with the combination of immune-body with the receptors of the red corpuscles. If the anti-immune-body had prevented the union of immune-body with the receptors of the red corpuscles, then we should have found that in the series treated with anti-immune-body more immune-body would have to be added subsequently before one dose remained free than was necessary in the series treated with immune-body alone.

On the combination of natural immune-bodies with anti-immune-body.

In view of the general law that an anti-substance developed in an animal combines with the substance introduced, and in view of the fact that the serum of the normal rabbit is used for injection, we would expect that bodies in this serum would unite with the anti-immune-body developed as the result of the injection. Bordet surmised that the anti-immune-body was the result of the introduction of the natural immune-bodies in the rabbit's serum, of which doubtless there is a large number, and Ehrlich and Sachs are decidedly of the same opinion. The combining properties of these immune-bodies might theoretically be demonstrated in two ways, viz. (*a*) by allowing them to combine with the anti-immune-body and thus interfering with its ordinary action, and (*b*) by allowing them to act on the anti-immune-body after it has combined with the red corpuscles treated with immune-body, and removing in part its inhibitory action. Both methods have been carried out.

(*a*) The direct combination of the anti-IB with the natural immune-bodies. The following is the scheme of experiment:

			W
A.	Anti-IB + rabbit's serum 55° (natural IBs)	+ $\overline{\text{RCs} + 5\ \text{IB}}$ washed	estimate how much C can then be taken up.
B (control).	Anti-IB + guinea-pig's serum 55°	+ $\overline{\text{RCs} + 5\ \text{IB}}$ washed	Do. do.

Example. In A the amount of C taken up was ·009 c.c.
In B „ „ „ „ „ ·014 c.c.

As half tubes (·5 c.c. of suspension of red corpuscles) were used, and the dose of C for half a tube was ·0037; there is a difference of fully a dose of C in the two series, *i.e.* the natural immune-bodies have united with an amount of anti-immune-body which would keep out more than a dose of C. Other experiments in which ox's serum 55° and simple salt solution were used in the control instead of guinea-pig's serum 55°, gave the same results. When rabbit's serum 55° is used, the diminished action of anti-immune-body (owing to its combination with the natural immune-bodies) is also shown by the much greater rapidity of lysis than is the case in the control.

We therefore see that of *the three heated sera tested the rabbit's is the only one which contains molecules capable of combining directly with anti-immune-body and thus of diminishing its action.*

The experiment quoted as an example was the one which showed most prominently the direct combination of the natural immune-bodies with anti-immune-body; in certain others it was practically imperceptible, though its influence on the rapidity of lysis was quite apparent. On the whole the results of the action of the natural immune-bodies in dissociating the anti-immune-body, now to be recorded, are more striking. The reason of this will be discussed below.

The dissociation of anti-immune-body by the corresponding natural immune-bodies.

Bordet found that the normal rabbit serum 55° (containing natural immune-bodies) had the power of separating anti-immune-body after it had combined with red corpuscles treated with immune-body. We have obtained in the case of our anti-immune-body results similar to those of Bordet and of striking character. The principle of the method of demonstration is simply this:—we take two series of tubes containing the same amounts of red corpuscles, of IB and of anti-IB; and after

time for combination is allowed the corpuscles are centrifugalised and washed. To those of one series normal rabbit serum 55° is added, to those of the other series guinea-pig's serum 55°, as a control. We then test how much complement can be taken up by the tubes of the two series. The following is the scheme:

		W	*W*	*W*
A.	$\overline{RCs+5\ IB}$	+anti-IB	+·3 c.c. rabbit's serum 55°	find how much C is taken up.
B (control).	$\overline{RCs+5\ IB}$	+anti-IB	+·3 c.c. guinea-pig's serum 55°	Do. do.

The average result may be said to be that treatment with ·3 c.c. rabbit's serum 55° had the effect of displacing sufficient anti-IB to allow the combination of fully two doses of complement.

In the control we have in other experiments replaced the guinea-pig's serum 55° by ox's serum 55° and also by salt solution. In all the experiments the same result has been obtained, viz. the treatment with rabbit's serum 55° *increases the power of taking up complement, i.e.* turns anti-IB out of combination with $\overline{RCs+IB}$.

The dissociation of anti-immune-body by the natural immune-bodies is thus amply proved; and as we stated above the result is more striking than that supplied by the experiment showing the prevention of combination of anti-immune-body by the natural immune-bodies. We cannot say definitely why this should be the case; but a possible explanation might be offered by supposing that there was an excess of anti-immune-body in the *preventive* experiment, so that a portion of it was free to combine with the red corpuscles united with immune-body (RCs + IB) and thus its effect was only slightly diminished. On the other hand in the *dissociation* experiment all the anti-immune-body is in the first place united with the corpuscles treated with immune-body, and the bringing of a large amount of immune-bodies (natural) into relation with it effects the separation of a considerable quantity. We have not been able so far to test this hypothesis by varying the amounts of anti-immune-body. The results, however, of the various experiments both on the prevention of union of anti-immune-body and also on its dissociation, were quite in accord in the two series.

On the prevention of combination of anti-immune-body by means of complement.

This is of course the converse to the experiments recorded above with regard to the keeping out of complement by means of anti-immune-

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body. Our experiments were carried out by heating two equal quantities (A and B) of red corpuscles at 55° C. over night as described above, then adding to both the same amounts of immune-body, whilst to those in A a large amount of complement is also added. After time for combination the corpuscles were washed and the fluid pipetted off. The two sets of corpuscles were then added to the same amounts of anti-immune-body, and then the amount of anti-immune-body in the supernatant fluid was tested for in the usual way. The results of several experiments all agreed in showing no distinct difference in the amounts of anti-immune-body taken up. We were thus unable to show that complement kept out anti-immune-body—a result which would have been practically conclusive as to the union of these two substances with the same combining-group.

As stated in the introduction—Bordet considered that the results which he obtained with regard to anti-immune-bodies, and which in all important points we have been able to confirm, were inconsistent with Ehrlich's side-chain theory, and especially his view regarding the amboceptor constitution of immune-bodies. His criticism, however, is based on the assumption that an anti-immune-body must combine with the cytophile group of the amboceptor, that is, must have the same combining-group as the corresponding cell-receptor. The facts demonstrated of course show that this is not the case. The combination of anti-immune-body with immune-body in no way interferes with the combination of the latter with the cell-receptor, and, as Bordet himself showed, the anti-immune-body acts by preventing the union of complement. As Ehrlich and Sachs state, however, the objections brought forward by Bordet entirely lose their force if it is maintained that the anti-immune-body unites with the complementophile group of immune-body. They hold that the immune-bodies of a given species have the same complementophile apparatus, and that it is a matter of indifference which immune-body is used to produce the anti-immune-body. The latter will act on all the immune-bodies with the same complementophile groups. It must be granted that the facts recorded with regard to anti-immune-bodies appear to support Ehrlich's hypothesis; whilst Bordet offers no theoretical explanation of the facts demonstrated. What is clearly established is that the anti-immune-body unites with a combining-group of the immune-body which is not

the cytophile group, and that this union prevents the union of complement with the complex immune-body + receptor. From the point of view of the animal injected with immune-bodies the development of the anti-immune-body may be regarded as a mechanism for preventing the associated action of the immune-bodies and its complement (though the action of other complements is also annulled).

This may be due to the anti-immune-body occupying the complementophile group of the immune-bodies; but that is not absolutely proved. When we consider the combining relationships of the different substances certain difficulties arise which have not yet received an explanation. As shown in a former paper, rabbit's and guinea-pig's complements combine with the same group in lysis by means of an immune-body from the rabbit, each keeping out the other from combination. The same holds with regard to an immune-body from the guinea-pig. According to the lock and key analogy the complementophile groups of the immune-bodies of the rabbit and the guinea-pig so far as they are satisfied by the same complements would thus appear to be the same. As, however, the anti-immune-body to the rabbit's immune-body does not act on the guinea-pig's immune-bodies the complementophile groups would appear to be different. It may also be remarked that a somewhat similar contradiction apparently existed in the anti-complement to the rabbit's complement not acting on guinea-pig's complement, while both complements showed the same combining affinities in lysis, *i.e.* in one instance they had apparently different combining groups, while in the other the groups were similar. In the case of the anti-complement we suggested[1] that the apparent contradiction might depend on the different energy of combination in the two instances, and possibly a similar explanation might hold here, as undoubtedly the union of anti-immune-body is a comparatively loose one.

The following is a summary of the results arrived at:

A. With regard to the haemolytic dosage of complement in different media:

(1) The dosage of complement varies greatly according to the medium in which the red corpuscles are suspended.

(2) The most striking variation observed was the very high dose of guinea-pig's complement necessary to produce lysis when ox's corpuscles are suspended in guinea-pig's serum 55°, a dose which is

[1] Muir and Browning, *loc. cit.*

about six times the dose necessary in salt solution and about three times the dose in ox's serum 55°.

(3) The high dose of complement necessary is chiefly due to the complement being prevented from entering into combination with the corpuscles treated with immune-body.

(4) When the complement in guinea-pig's serum is removed by combination instead of being converted into complementoid by heating at 55° C., the dosage of complement in such a treated serum is much diminished and becomes approximately the same as in ox's serum 55°.

(5) We conclude that the presence of a large amount of guinea-pig's complementoid interferes with the combination of complement, and the dose of the latter necessary for lysis is thus increased.

B. With regard to anti-immune-bodies (the results of course apply only to those studied by haemolytic methods):

(1) The anti-immune-body obtained by injecting the normal serum of the rabbit was shown to act on two immune-bodies produced in the rabbit by active immunisation, and the corresponding anti-immune-body obtained by injecting guinea-pig's serum was shown to act on two natural immune-bodies in the serum of that animal.

(2) The anti-immune-body is shown by quantitative experiments not to interfere in any way with the combination of immune-body with the receptors of the red corpuscles.

(3) The anti-immune-body combines with red corpuscles treated with immune-body and interferes with the combination of complement. Lysis may thus be prevented by the anti-immune-body; but where this is not the case it is shown by quantitative methods that a certain amount of complement is kept out of combination.

(4) The natural immune-bodies of the serum injected combine directly with the anti-immune-body developed, and have also the power of dissociating anti-immune-body after it has combined with immune-body fixed to red corpuscles. The combination of anti-immune-body is a comparatively weak one and belongs to the group of reversible actions.

(5) We have been unable so far to demonstrate that the previous combination of complement prevents the union of anti-immune-body with red corpuscles treated with immune-body.