ON THE DEVIATION OF COMPLEMENT BY A SERUM AND ITS ANTI-SERUM AND ITS RELATIONS TO THE PRECIPITIN TEST.

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Recent researches on the subject of immunity, and especially those dealing with haemolytic sera have thrown a flood of light on the complicated constitution of serum and other fluids and have given us the means of attacking problems which were, and still are, quite inaccessible to ordinary chemical methods. It is now well known that when bacteria or the cells of another species of animal are injected into an animal, there are developed immune-bodies which in association with complement or alexine produce the bactericidal or haemolytic effect. From the point of view of chemical combination the all-important fact is that certain molecules or receptors in bacteria, etc. give rise to anti-substances which lead to the fixation or absorption of complement. It has also been shown that in a normal serum in addition to complement there are also present the homologues of receptors and immune-bodies. Accordingly when a serum of one animal is injected into another of different species there is theoretically the possibility of the development of three different kinds of anti-substances. Anti-complements have received most attention hitherto, as their action was the first to be recognised, but recently their existence has been called in question, as will presently be explained. The subject of anti-immune-bodies, produced by the injection of a normal serum, has recently been treated of in this *Journal* by one of us in collaboration with Dr Browning[1], and

[1] Muir and Browning, *Journal of Hygiene*, 1906, p. 1.

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their mode of action has been detailed. In the present paper we propose to deal with the anti-substances developed by the receptors of a normal serum, these two bodies in conjunction leading to the absorption or fixation of complement. We shall in the first place refer to the work which has recently been done on this subject.

In 1902 Gengou[1] showed that when an anti-serum was developed by the injection of various albuminoid substances into an animal, the mixture of the substance and the anti-substance might not only give rise to a precipitate, but might also have the power of absorbing complement or alexine. This phenomenon he regarded as analogous to what was known to obtain with regard to haemolytic sera and bacteriolytic sera, and he spoke of the anti-substances developed in the treated animals as "sensibilisatrices (immune-bodies) contres les substances albuminoids." In Ehrlich's terminology we may express this by saying that the receptors in the albuminoid molecules gave rise to immune-bodies or amboceptors and that the combination of the two took up complement. Gengou obtained this result with milk, egg-white, fibrinogen and the serum of another species of animal than that injected. Special attention has recently been drawn to the subject by a paper by Moreschi[2] on the nature of anti-complements. An "anti-complement" obtained by injecting a normal serum acts chiefly, as is well known, on the serum ("complement") injected; but Moreschi has shewn that if a minute quantity of the homologous serum is added to the anti-serum various complements may be taken up, that is antagonised; or in other words the anti-serum behaves as an anti-complement to various complements. He points out that an extremely minute quantity, (·000,01 c.c.) of the original normal serum added to the anti-serum may result in the taking up or deviation of various complements. From his experiments he has found reason to doubt whether there is any real anti-complement in the strict sense, *i.e.* a substance which unites directly with a complement and thus prevents its action, the apparently anti-complement action being a deviation phenomenon. Neisser and Sachs[3] have applied Moreschi's results to the differentiation of the bloods of different species of animals, and have shown that the deviation of complement is a much more delicate test than the precipitation test; that is, a much smaller amount of the serum (precipitinogen) when added to the anti-serum (precipitin) will produce a deviation or

[1] Gengou, *Annales de l'Inst. Pasteur*, 1902, p. 734.
[2] Moreschi, *Berlin. klin. Wochenschr.* 1905, p. 1181.
[3] Neisser and Sachs, *Berlin. klin. Wochenschr.* 1905, No. 44, and 1906, No. 3.

fixation of complement, than that necessary to cause a visible precipitate. They consider that the phenomenon is analogous to the fixation of complement by a cell-receptor when combined with its corresponding amboceptor. Gay[1] regards the precipitate as the all-important factor in the fixation of complement and finds that when a precipitate forms the separated fluid is without effect on complement, whilst the precipitate fixes, or combines with a considerable quantity. He extends his observations with the purpose of showing that phenomenon of deviation in haemolytic and bactericidal experiments may depend upon fixation by precipitate. Pfeiffer and Moreschi[2] have found that the precipitate, by fixing complement, has an anti-bacteriolytic action in the animal body. In a second communication on anti-complements Moreschi[3] considers the quantitative relationships between the serum and anti-serum (precipitinogen and precipitin) in relation to the absorption of complement, and comes to the conclusion that these unite in variable proportions. He also emphasizes the parallelism between the amount of precipitate formed and the amount of complement (alexine) absorbed. Friedberger[4] gives an account of his observations on this subject, one of the most important of which is that, while most of his anti-sera have shown deviating powers similar to those recorded by others and observed by ourselves, he has obtained an anti-human serum which gives a deviation in the extraordinarily minute amount of ·000,000,001 c.c. Even ·000,01 c.c. of human sweat produced a recognisable fixation of complement with this anti-serum. This is further referred to below. The subject is critically reviewed by Liefmann[5] especially in relation to precipitum-formation on the one hand and amboceptor-action on the other. He considers that a satisfactory explanation of the fixation of complement is not yet possible.

It thus appears that a number of questions, both of practical and of theoretical importance, are opened up by these investigations and we shall deal with some of them in the present communication.

Methods. The method which we used of preparing the anti-serum is that usually followed in precipitin work, viz.: the intra-peritoneal injection of a particular serum in varying doses at suitable intervals

[1] Gay, *Centralbl. f. Bakteriol.* 1905, xxxix. Abtheil I. Originale, p. 603; *Annales de l'Inst. Pasteur*, 1905, p. 593.

[2] Pfeiffer and Moreschi, *Berlin. klin. Wochenschr.* 1906, p. 33.

[3] Moreschi, *ibid.* 1906, p. 76.

[4] Friedberger, *Deutsche med. Wochenschr.* 1906, p. 578.

[5] Liefmann, *Berlin. klin. Wochenschr.* 1906, p. 448.

of time. The anti-serum before being used is of course heated at 55° C. to destroy its complement. We have used three anti-sera obtained from the rabbit, which act on the serum of man, the ox, and the guinea-pig, respectively, and one from the guinea-pig acting on rabbit's serum. We may conveniently represent the first of these anti-sera as *anti-serum rabbit v. man*, and so with the others. The method of testing the deviation of complement is carried out in two stages. (1) To each of a series of test tubes a given amount of the anti-serum, usually ·05 c.c., is added along with a given amount of the homologous serum, and the volume is made up with ·8 per cent solution of sodium chloride to 1 c.c. To the several tubes varying amounts of complement (normal serum of rabbit or guinea-pig) are added, the smallest amount being about the minimum haemolytic dose for the amount of red corpuscles to be afterwards used in the test. It will be seen that in this stage there is always a mixture of three substances, the serum, the anti-serum, and the complement whose absorption is to be observed. The tubes are placed in the incubator at 37° C. for $1\frac{1}{2}$ to 2 hours to allow time for combination. (2) At the end of that time 1 c.c. of a 5 per cent. suspension of red corpuscles treated with the corresponding immune-body is added to each tube and the tubes are again placed in the incubator for $1\frac{1}{2}$ hours. We can thus observe the tube in which there is the first trace of lysis, and the tube in which lysis is first complete. It is convenient to prepare several series of tubes with different amounts of the homologous serum, ·01, ·001, ·000,1 c.c., etc. In each experiment a control series with anti-serum along with different amounts of complement alone is prepared, and the difference between the lysis in this and the other series is interpreted as being due to the presence of the homologous serum. We also have in each series a tube containing corresponding amounts of serum and anti-serum alone, to show precipitation. It is evident that the conditions of experiment may be varied by making the amount of the homologous serum (precipitinogen) fixed, and varying the amount of anti-serum.

It is thus seen that we have in such experiments two substances and their anti-substances, viz.: serum + anti-serum and red corpuscles + immune-body: opportunity is given to the complement to unite with the first combination and then the presence of free complement is tested for by its haemolytic effect.

1. *Phenomena of Deviation of Complement.*

The following may be taken as a typical example of a deviation experiment.

Anti-serum, rabbit *v.* ox, ·05 c.c. to each tube.
Serum of ox 55° C.[1], ·01 – ·000,001 c.c.
Deviation of guinea-pig's complement.
Test for complement = 1 c.c. suspension of ox corpuscles + immune-body.
Minimum haemolytic dose of complement = ·01 c.c.

TABLE 1.

Anti-serum c.c.	Ox-serum 55° C. c.c.	Amounts of complement ·01	·02	·03	·04	·05	·06 c.c.	
·05	0	$\frac{7}{10}$	complete	complete	complete	complete	complete	Amount of lysis in added corpuscles
·05	·000,001	$\frac{2}{5}$	almost complete	complete	complete	complete	complete	
·05	·000,01	trace	$\frac{3}{4}$	just complete	complete	complete	complete	
·05	·000,1	0	$\frac{1}{2}$	$\frac{3}{4}$	nearly complete	complete	complete	
·05	·001	0	0	0	0	slight trace	trace	
·05	·01	0	0	0	0	0	0	

The amount of lysis indicated in this and other tables is of course due to the amount of complement left free after contact with the serum and its anti-serum for $1\frac{1}{2}$ hours at 37° C. The results of this experiment are graphically represented in the accompanying figure (Fig. 1, p. 270).

It will be seen from the amounts of the resulting lysis given in the table that without any of the ox's serum ·01 c.c. of complement gives $\frac{7}{10}$ lysis of the added corpuscles, whilst the addition of ·000,001 c.c. of ox serum reduces the lysis to $\frac{4}{10}$; that is, this amount of serum in combination with the anti-serum has deviated about a third of a dose of complement. With ·001 c.c. of serum six haemolytic doses of complement produce only a trace of lysis. It is also to be noted that if we take the first tube in the series where any lysis is present, much more than a dose of complement must be added before lysis is complete: for example, with ·0001 c.c. of serum two doses must be added in order that lysis may be complete. If we suppose that a new body is formed by the union of a molecule in the serum with one in the anti-serum, then the combination of this new body with complement presents phenomena well recognised

[1] In each case the serum used is heated to 55° C. for an hour at least, to destroy the complement naturally present.

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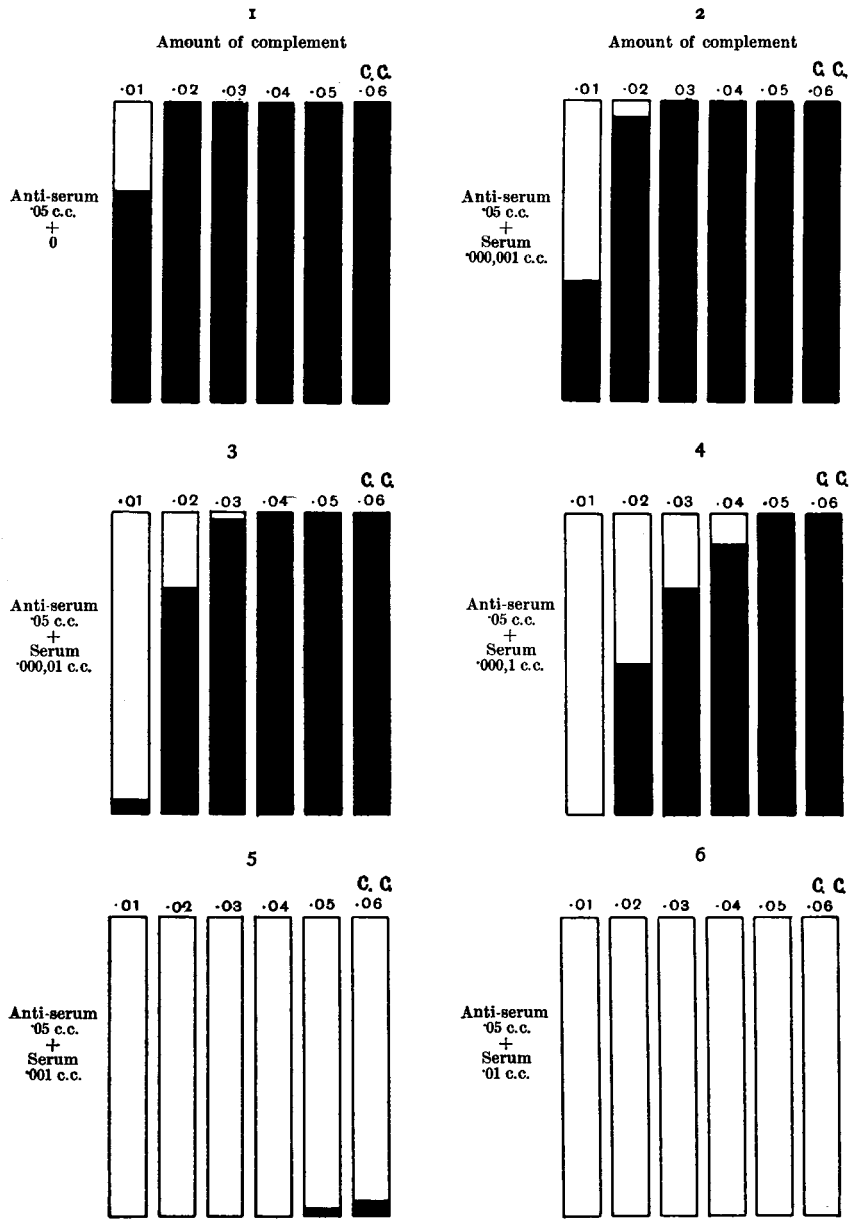

Fig. 1. This figure represents diagrammatically the results of the experiment given in Table 1. The columns indicate test tubes, and the height of the black portion shows the amount of lysis of the test corpuscles in each. The amounts of serum, anti-serum, and complement are also given.

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to obtain in the case of the union of toxin and anti-toxin—the so-called "Ehrlich's phenomenon."

The table also shows that increasing amounts of serum lead to the taking up of more complement, though this does not occur in arithmetical proportion. This point will however be referred to below.

The following table shows the result of a similar experiment with anti-human serum.

Anti-serum rabbit *v.* man, ·05 c.c. to each tube.
Test for complement = ·5 c.c. suspension of ox's corpuscles treated with immune-body.

TABLE 2.

Anti-serum c.c.	Human serum c.c.	Rabbit's complement ·05	·1	·2	·3 c.c.	
·05	0	practically complete	complete	complete	complete	Lysis in added corpuscles
·05	·000,01	very slight	just complete	complete	complete	
·05	·000,1	none	none	$\frac{1}{5}$	complete	
·05	·001	none	none	none	none	
·05	·01	none	none	none	none	

From the above it will be seen that ·000,01 c.c. human serum produces distinct deviation. Precipitin tests were carried out at the same time with the result that ·000,1 c.c. gave only a very slight, in fact rather doubtful precipitate, whilst ·000,01 c.c. gave no trace of precipitate.

Table 3 shows the action of our third serum.

Anti-serum, rabbit *v.* guinea-pig.
Test for complement = ·5 c.c. suspension of ox's corpuscles treated with immune-body.

TABLE 3.

Anti-serum c.c.	Guinea-pig's serum c.c.	Rabbit's complement ·05	·075	·1	·15 c.c.	
·04	0	0	0	almost complete	complete	Lysis in added corpuscles
·04	·000,01	0	0	0	0	
·04	·000,1	0	0	0	0	

In this case the anti-serum alone has a slight though distinct effect on the haemolytic action of rabbit's complement; this is probably due to the fact that rabbit's and guinea-pig's sera contain a few common receptors. The addition of even ·000,01 c.c. of guinea-pig's serum 55° however gives a marked deviation phenomenon. This experiment is of special interest as the anti-serum used gave practically no precipitate when added to the homologous serum; even ·01 c.c. of the latter produced merely a faint cloudiness but no real precipitate.

2. *On the Deviation of different Complements.*

It can be readily tested by the above methods whether or not any given complement is taken up by a particular combination of serum + anti-serum. Moreschi found that a number of different complements may be deviated by the same combination, or as he expresses it, an anti-serum becomes anti-complement to the complements of different animals on the addition of a small quantity of the homologous serum. Our observations are confirmatory of this, but they show also that some complements may not be deviated. The red corpuscles used in testing for complement may be treated with an immune-body artificially developed for these corpuscles, or we may depend in some instances on the natural lysis which may be produced by a normal serum. (In this latter case there is of course in the serum a natural immune-body or Zwischenkörper which acts along with the complement.) The following may be cited as examples. The anti-serum rabbit *v.* ox (along with the homologous serum) deviates (*a*) guinea-pig's complement as tested either with ox's or rabbit's corpuscles treated with the corresponding immune-body, and (*b*) rabbit's complement when tested in the same way; it also deviates (*c*) dog's complement as tested by the natural lysis of dog's serum on rabbit's corpuscles. The anti-serum rabbit *v.* man deviates both (*a*) rabbit's and (*b*) guinea-pig's complement when tested with ox's corpuscles treated with immune-body, and also (*c*) cat's complement when tested by the natural lysis of guinea-pig's corpuscles by cat's serum. If we regard the specific substance in the anti-serum as the homologue of an immune-body, these results show that many complements are taken up through the medium of the same immune-body. Analogous results are obtained in the case of haemolytic immune-bodies.

We have however met with the following two exceptions, though we have made no very extended series of observations, and probably many others will be found to obtain. The anti-serum rabbit *v.* ox (along with the homologous serum) does not deviate ox's complement in the natural lysis of rabbit's corpuscles by ox's serum. Again, the anti-serum rabbit *v.* guinea-pig does not deviate rabbit's complement when guinea-pig's corpuscles treated with immune-body from the rabbit, are used as the test[1]. *It is thus shown that many, but not all, complements are taken up by the combination of a serum with its anti-serum.*

[1] In this case there is a striking analogy to what Muir and Browning (*Proc. Roy. Soc. Lond.* 1904, vol. LXXIV. p. 298) found in the case of haemolytic immune-bodies, viz.: that

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Another point worthy of note is that we have observed an apparent variation in the firmness of union of the complement deviated. This is indicated by the manner in which the lysis progresses when the test corpuscles are added; in some cases the lysis comes to an end after an hour or an hour and a half at 37° C., in others it continues to increase, as if the complement were being separated from the combination of serum + anti-serum molecules. For example, with the anti-serum rabbit *v.* ox along with the homologous serum, the combination of guinea-pig's complement appeared to be firmer than that of rabbit's complement, whereas with the anti-serum rabbit *v.* man the converse was the case. The results, in short, point to the possibility in some cases of complement becoming dissociated from the combination serum + anti-serum, or in other words the deviation of complement may exhibit varying degrees of permanency.

3. *Deviating Power as compared with Haemolytic Action.*

As already stated, the union of the molecules in the anti-serum with those in the homologous serum leads to the taking up of complement. In this we have a close analogy to what occurs in the case of a haemolytic serum, where by the union of the receptors of the red corpuscles with immune-body, complement enters into combination, this combination as one of us showed, being generally of firm nature. Apparently then when a serum is injected into an animal, there are formed molecules which are the homologues of immune-bodies. The amount of serum along with its anti-serum necessary to produce deviation is however, out of all proportion smaller than the amount of red corpuscles (treated with immune-body) necessary to show an appreciable absorption of complement. There are also differences to be mentioned below when the combination of the molecules and anti-molecules are used in varying proportions in the two cases. It has been shown by Morgenroth[1] that a haemolytic serum may be developed by the injection of serum, the latter apparently containing receptors with the same combining group as the haemolytic receptors of the red corpuscles. The anti-serum

increased amounts of immune-body for guinea-pig's corpuscles did not take up (or deviate) increased amounts of rabbit's complement when guinea-pig's corpuscles + immune-body were used as the test, whereas they did so when ox's corpuscles + immune-body were used. In fact if we substitute anti-serum to guinea-pig's serum for immune-body to guinea-pig's corpuscles the results coincide in the two cases.

[1] Morgenroth, *München. med. Wochenschr.* No. 25, 1902.

to ox's serum used by us has haemolytic action, the minimum haemolytic dose for ox's corpuscles being ·05 c.c.; as already stated, it gives deviation of complement with ·000,001 c.c. of ox's serum. The haemolytic serum acting on ox's corpuscles has a minimum haemolytic dose of ·0015: it also gives deviation along with ox's serum, but not with a smaller dose than ·001 c.c. The former anti-serum has thus only about a thirtieth of the haemolytic action of the latter, but has about a thousand times more deviating power when tested with the homologous serum. Furthermore, if the anti-serum to ox's serum be left for a time in contact with a sufficient amount of ox's corpuscles, practically all the haemolytic immune-body can be removed, but the precipitating and deviating properties remain in the serum. It is thus evident that the molecules in the serum which, in association with the anti-serum, deviate complement in such experiments are different from the haemolytic receptors[1].

4. *Relation of the Deviation of Complement to Precipitation.*

That the union of the two substances concerned in the fixation of complement is often attended with precipitation has been recognised by various observers. Moreschi in his first paper states that the deviation phenomenon appears only as a sequel to precipitation and stands in closest relation to it; in his second publication however he speaks less decidedly on this point, though he says that the amount of complement fixed is always in proportion to the amount of precipitate. Neisser and Sachs in describing the application of the deviation test for differentiating the blood of different species regard the results by the two methods as analogous, but consider that the essential in the phenomenon of deviation is the union of a substance and its anti-substance (amboceptor). Gay speaks of the "fixation of alexins by specific serum precipitates" and finds, as stated above, that the precipitate separated and washed takes up complement. We shall first state the facts which we have observed, and afterwards consider their significance.

[1] *Note.* Gay has suggested that probably many errors have arisen in haemolytic experiments through non-recognition of the deviation of complement by a serum + its anti-serum, and has pointed out the difficulty in freeing red corpuscles completely from the serum by washing. Even however if a small quantity of serum be left the amount of anti-serum necessary to produce deviation is relatively great, and as all the haemolytic sera which I have used have been powerful (·003 c.c. being generally the haemolytic dose) I am certain that no error of importance can have arisen from this cause in the work which I have published. Our views regarding anti-complements however require revision in view of the results established with regard to deviation of complements. R. M.

In the first place we may consider the relative delicacy of the two reactions. All observers who have written on the subject state that the deviation test is more delicate than the precipitin test, and our results agree with this. In making the comparison the occurrence of precipitation is observed by using the same amount of anti-serum (generally ·05 c.c.) in each case along with varying amounts ·01, ·000,1, ·000,01, c.c. etc. of the homologous serum, the volume being made up with salt solution to 1 c.c. The tubes are placed in the incubator for 1½ hours as in the deviation experiments, and then in the refrigerator till next morning, when the results are read off. With ·05 c.c. of our anti-serum rabbit *v.* ox a distinct precipitate is got on the addition of ·001 c.c. of ox serum, a very faint precipitate with ·000,1 c.c., this latter being scarcely reliable for practical purposes. Distinct deviation of rabbit's and guinea-pig's complement is got with ·000,01 c.c. of ox serum and appreciable deviation sometimes even with ·000,001 c.c. With the serum rabbit *v.* man ·05 c.c. precipitation is distinct with ·001 c.c. of human serum, almost absent with ·000,1 c.c.; deviation of complement is always got with ·000,01 c.c. We may thus state that in the case of these two sera the deviation test is between ten and a hundred times more delicate than the precipitin test. Neisser and Sachs in the case of an anti-human serum considered the deviation test was about forty times more delicate, and Friedberger found an even greater difference between the two. The figures which we have stated may be taken as well within the limits, as we have taken the smallest amount of serum which gives a *distinct* deviation. Furthermore, owing to the nature of the reaction the result is much more easily appreciated than in the case of precipitins, especially when there is any natural cloudiness of the serum.

We have also found that the phenomenon of deviation may be well marked in the case of an anti-serum which gives no precipitate. Nuttall states that when the animal used for injection is of closely allied species to that from which the serum is taken a precipitin is not usually developed. We have obtained a result which confirms this in the case of the anti-serum rabbit *v.* guinea-pig. This anti-serum produces no distinct precipitate even when a comparatively large amount—*e.g.* ·01 c.c. of the homologous serum is added; at the most there is only some opalescence, and if the tubes be allowed to stand for twenty-four hours there is no distinct deposit. It will be seen from Table 3 that with this anti-serum, which produces no precipitate on addition to the homologous serum, the phenomenon of deviation is

produced by exceedingly small amounts of the latter, viz. ·000,01 c.c. This shows that the injection of the guinea-pig's serum into the rabbit gives rise to anti-substances, although the latter cannot be demonstrated by the phenomenon of precipitation. We also found in the course of immunising a rabbit against human serum that the deviating power appeared before any precipitating action was detectable. We may state however, that when a precipitate is formed we have always found that it had the property of fixing complement—the test of course being made after the precipitate had been washed and centrifugalised several times. In fact the use of a separated precipitate supplies a very convenient method for freeing a serum of complement. We have, for example, been able by this means to deprive guinea-pig's complement of practically all haemolytic power for ox's corpuscles treated with immune-body, though the haemolytic dose of such a complement for 1 c.c. of suspension of red corpuscles may be as low as ·01 c.c. That the precipitate when formed, or rather molecules in the precipitate, have the power of fixing complement, there is therefore no doubt. Are there any molecules with this property in the separated fluid? To each of a series of tubes ·025 c.c. anti-serum rabbit *v.* ox and ·001 c.c. of ox serum were added, the mixture being made up to 1 c.c. in each tube with salt solution. Another similar series was prepared with ·01 c.c. ox serum instead of ·001 c.c. The tubes were incubated for two hours and then placed in the refrigerator till next morning. The tubes were then centrifugalised and the supernatant fluid was carefully pipetted off from the precipitate in each tube. To the fluid thus obtained different doses of guinea-pig's complement were added and after incubation deviation was tested for in the usual way. The result in both series was negative, *i.e.* the molecules which fix complement were practically all in the precipitate. In another experiment with ·01 ox serum and ·025 anti-serum, performed in the same way, we found that the supernatant fluid deviated about one-twelfth of a haemolytic dose of guinea-pig's complement—an exceedingly small amount. So also in the case of anti-human serum we found that the precipitate obtained by mixing ·1 c.c. of human serum with 1 c.c. of the anti-serum in 5 c.c. of salt solution, possessed exclusively deviating power, the separated fluid being practically without any effect when tested. These results are in harmony with those obtained by Gay on this point.

We may also add that there is no question of complement being carried down mechanically by the precipitate in process of formation.

The precipitate after it has formed may be repeatedly washed and still retains the property of fixing complement.

Observations on the relation between precipitate formed and the deviating power show that the amounts are not strictly proportional. For example, using ·025 c.c. of anti-ox serum along with varying amounts (·1, ·01 c.c. etc.) of ox's serum, we found that the maximum deviation of complement was given by ·001 c.c., whilst distinctly the greatest amount of precipitate was given by ·01 c.c. Again, on using the same quantity of serum, viz. ·001 and varying the amounts of anti-serum we found that a much greater amount of precipitate was given by ·3 c.c. of anti-serum, than by ·1 c.c., whilst the amount of complement fixed was practically the same in the two cases. Further details on this point are given below under Section 6. It is also interesting to note that Friedberger and Liefmann have found that it is possible by heating to deprive an anti-serum of its precipitating action while its power of fixing complement in association with the homologous serum may be retained.

We therefore conclude that (1) when a precipitate forms, the deviating property is contained in it, and may be so exclusively; on the other hand (2) the deviation phenomenon may occur without precipitation and (3) the amount of deviation is not always in proportion to the amount of precipitate. The last-mentioned fact would indicate, as Moreschi suggests, that the precipitate has not always the same composition, and possibly the precipitin and the precipitinogen unite in varying proportions.

5. *The Deviation of Complement as regards Specificity.*

The important practical question, with regard to the deviation of complement, is the same as in the case of precipitins, and concerns the possibility of distinguishing different kinds of bloods, or rather sera. We have carried out a number of observations on this subject, though these must be regarded as of a preliminary nature, and a much more extended series will be necessary. Using an anti-human serum, we have tested the sera of various animals with it, and observed whether there was any deviation of rabbit's complement (ox's corpuscles treated with immune-body from the rabbit being used as the indicator). We have obtained purely negative results with the sera of the ox, sheep, pig, dog, cat, mouse, guinea-pig, horse, and pigeon. In each case ·05 c.c. of the anti-serum was used along with as much as ·01 c.c. of the serum to be tested: in every case as much complement was found to be free as

when the anti-serum was used alone. In the case of the primates however, distinct deviation of complement was obtained. With the serum of a chimpanzee, for which we are indebted to Prof. Woodhead, the following are the results obtained, the details being as in former tables:—

Human anti-serum ·05 c.c. to each tube.

The indicator was ·5 c.c. suspension of red corpuscles of ox treated with immune-body from rabbit.

TABLE 4.

Anti-serum c.c.	Chimpanzee serum c.c.	Complement of rabbit ·05	·1	·2 c.c.	
·05	0	just complete	complete	complete	Lysis in added corpuscles
·05	·001	none	half lysis	complete	
·05	·000,1	none	complete	complete	
·05	·000,01	just complete	complete	complete	

It is thus seen that ·000,01 c.c. gives no perceptible deviation, whilst ·000,1 c.c. deviates a haemolytic dose. In a corresponding test made with human serum, it was found that ·000,01 c.c. of human serum produced the same deviation as ·000,1 c.c. of chimpanzee serum. We have also tested the serum of a monkey, viz. *Macacus rhesus* for several samples of whose blood we are indebted to Mr Jolly, of the Physiological Department of the University of Edinburgh, and were rather surprised to find that it had practically the same deviating power as the chimpanzee serum. Several tests were made, and on every occasion with similar result. Neisser and Sachs also obtained negative results with the bloods of the rat, pig, goat, rabbit, ox, and horse, when these were tested with an anti-human serum. With monkey's serum an interference with haemolysis was however obtained, the amounts of monkey's serum being rather more than ten times the amount of human serum necessary to obtain the same result: the species of monkey is not stated.

With regard to precipitates, the results of observations made at the same time are shown in the following table.

Anti-human serum ·05 c.c. to each tube.

TABLE 5.

Amount of serum	Human serum	Chimpanzee serum	Macacus serum
·000,1 c.c.	? Slight opalescence	0	0
·001	Distinct precipitate	Slight but distinct	? Slight opalescence
·01	Marked	Marked, less than with human	Distinct though slight

These results are much in conformity with what Nuttall[1] obtained.

Whilst, however, the deviation test places the chimpanzee and the macacus monkey in practically the same relation to man, the precipitation test brings out a difference, the chimpanzee serum giving a more marked reaction with anti-human serum than the macacus serum does. No doubt other analogous results will be found to obtain.

We have also tested the *anti-ox serum* with the sera of some other animals. Using ·01 c.c. and ·001 c.c. of the serum to be tested, we obtained no deviation with the serum of the horse, pig, cat, mouse, pigeon and of man. (Larger amounts of serum than those mentioned were not used, as complications may arise from the added serum interfering with lysis.) With the serum of the sheep, however, a deviation was obtained approximating in degree to that given by ox's serum. With ·05 c.c. anti-ox serum, ·000,01 c.c. sheep's serum as well as ·000,01 c.c. ox serum gave a slight though distinct deviation of guinea-pig's complement, but this was more marked in the case of the ox's serum. A greater difference was, however, brought out when we tested the amount of deviation with a larger amount of the two sera, viz. ·001 c.c. (the same quantity of anti-serum, ·05 c.c., being used). In this test we found that six haemolytic doses of guinea-pig's complement in the case of the ox serum and four haemolytic doses in the case of the sheep's serum, had to be added before one free dose was obtained. With the precipitin test analogous differences were obtained—a slight precipitate was given by ·000,1 c.c. of both sera, more distinct in the case of the ox serum; a doubtful trace of precipitate with ·000,01 c.c. of ox serum, and no precipitate with ·000,01 c.c. of sheep serum. These results also as regards precipitation are in accordance with those obtained by Nuttall. In the case, therefore, of ox and sheep serum as tested by anti-ox serum there is a close parallelism between results obtained by the deviation and the precipitation tests.

It may also be mentioned that of a number of sera tested with an anti-goat serum, Moreschi found that the only one, besides the goat's serum, which gave a deviation result was ox's serum.

Although our observations on this subject have been as yet comparatively restricted in scope, they have been sufficient to show a harmony in the results brought out by the two methods. It is quite likely that in a more extended enquiry, one method may, in certain instances, bring out differences with regard to blood relationships which the other fails to do (as for example we found in a com-

[1] Nuttall, *Blood Immunity and Relationship*, Cambridge, 1904, p. 165.

parison of the serum of the chimpanzee and the serum of the macacus monkey).

With regard to the application of the deviation to forensic purposes, we have practically nothing to add to what has been stated by Neisser and Sachs. It is an important adjuvant to the precipitin method, and will undoubtedly be of great service when there is any cloudiness in the fluid to be tested; the non-occurrence of haemolysis is a phenomenon so much more easily appreciated than the formation of a slight precipitate. Undoubtedly also the deviation method is considerably more delicate, and Friedberger in the paper above quoted points out that its extreme delicacy, when a very powerful anti-serum is obtained, may be a source of fallacy, as he has obtained reactions with human sweat. For this reason he advises the use of anti-sera which give deviations with ·000,01 c.c. of the homologous serum as a minimum; with a serum of this strength there is no risk of error such as might arise from the material to be tested being impregnated with sweat.

6. *On the Quantitative Relations of Serum and Anti-serum to the Deviation of Complement.*

In the tables given above it appears that the amount of deviation of complement increases, though not in arithmetic ratio, with the amount of serum, when the amount of anti-serum is kept constant. If, however, comparatively large amounts of the homologous serum be used, the amount of complement taken up again becomes diminished as the amount of serum increases. We have carried out a number of

Anti-serum, rabbit *v.* ox, ·025 c.c. to each tube, with varying amounts of ox's serum 55° C.

Test for complement = 1 c.c. suspension of ox's corpuscles treated with immune-body; the dose of complement for this is ·0075 c.c.

TABLE 6.

Anti-serum c.c.	Ox serum 55° C. c.c.	Guinea-pig's complement ·0075	·015	·03	·05	·07 c.c.	
·025	·1	very slight	$\frac{2}{5}$	complete	complete	complete	Lysis in added corpuscles
·025	·01	0	$\frac{1}{4}$	$\frac{3}{4}$	nearly complete	complete	
·025	·001	0	very slight	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{2}$	
·025	·000,1	0	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	just complete	
·025	·000,01	$\frac{1}{2}$	$\frac{2}{3}$	just complete	complete	complete	

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observations on this subject, one or two examples of which will exemplify the phenomenon. The results are confirmatory of those recently published by Moreschi, though the details of experiment are somewhat different.

It is thus seen that with a given quantity of anti-serum, ·025 c.c. in this case, there is an optimum amount of serum which gives the maximum deviation, viz. ·001 c.c., whilst above as well as below that optimum the amount of deviation diminishes. The maximum precipitate was given by ·01 c.c. The results of this experiment are graphically represented in Figure 2.

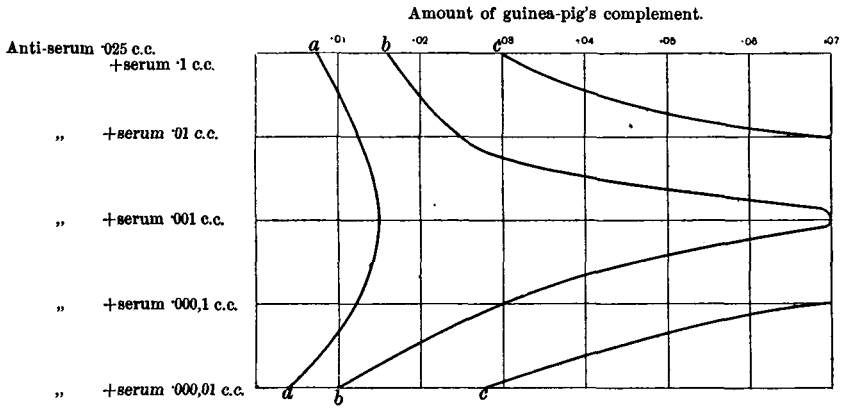

Fig. 2. This figure shows the amount of deviation of complement with ·025 c.c. of anti-serum and varying amounts of homologous serum. The maximum deviation is given with ·001 c.c.

curve a = initial lysis,
b = half lysis,
c = complete lysis.

A similar result was obtained with anti-human serum, as is shown in the following table.

Anti-serum rabbit *v.* man, ·025 c.c. to each tube. Varying amounts of human serum.

TABLE 7.

Anti-serum c.c.	Human serum 55°C. c.c.	Guinea-pig's complement ·01	·02	·03	·04	·05 c.c.	
·025	·1	—	—	complete	complete	complete	Lysis in added corpuscles
·025	·01	—	—	—	complete	complete	
·025	·001	—	—	—	—	complete	
·025	·000,1	—	—	complete	complete	complete	
·025	·000,01	—	complete	complete	complete	complete	

In this experiment there was some diffusion of haemogolobin owing

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to the corpuscles having been kept too long; the tubes showing *complete* lysis are accordingly only given. The maximum deviation was thus given by ·001 c.c. of serum. On the other hand ·01 c.c. gave the maximum precipitate.

We have shown above that complement may be fixed by a serum plus its anti-serum even although there be no precipitate. We have accordingly enquired whether in this instance the principle of optimum proportions holds, and have found that this is the case. The following may be taken in illustration:—

Anti-serum, rabbit *v.* guinea-pig, ·025 c.c. to each tube. Varying amounts of guinea-pig's serum.

Test for complement = ·5 c.c. suspension of ox's corpuscles treated with immune-body.

TABLE 8.

Anti-serum c.c.	Guinea-pig serum 55° C. c.c.	Rabbit's complement ·05	·1	·2	·3	·4 c.c.	
·025	·1	$\frac{1}{3}$	complete	complete	complete	complete	Lysis in added corpuscles
·025	·01	0	0	complete	complete	complete	
·025	·001	0	0	$\frac{1}{4}$	just complete	complete	
·025	·000,1	0	just complete	complete	complete	complete	
·025	·000,01	almost complete	complete	complete	complete	complete	

In this case also it will be seen that the maximum deviation occurs with ·001 serum, whilst above, as well as below this amount the deviation of complement diminishes.

It has been recognised by various observers in testing a precipitin (anti-serum) with a given amount of the homologous serum, that the precipitate may become less when the amount of serum is increased beyond a certain point, and after a precipitate has formed this may be dissolved on adding homologous serum. For example, with ·001 c.c. ox serum and ·05 c.c. anti-serum a bulky precipitate is obtained, but this is dissolved in great part on the addition of ·1 c.c. ox serum. In this respect also there is an analogy between the phenomena of precipitation and of deviation of complement. We cannot, however, agree with Moreschi when he says that the amount of complement fixed always depends upon the amount of precipitate, as the results above given show that the maxima of the two reactions may not correspond.

We may vary the conditions of experiment by keeping the amount of serum fixed and varying the amount of anti-serum. In this case Moreschi also found, as shown in his table No. 3, that on increasing the

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amount of the latter an optimum point was reached, beyond which additional increase of anti-serum resulted in diminution in the amount of complement taken up. We have made a large number of observations on this point, but with varying results. In one or two instances we found a slight diminution in the amount of complement deviated, as the anti-serum was increased, but this was never very marked; whilst in the majority of cases we found no such diminution, even when as much as ·3 c.c. anti-serum was added. At present we cannot give any explanation of this discrepancy. Certainly the phenomena of optimum deviation do not occur in the same striking manner as when the amount of anti-serum is kept fixed and the amount of homologous serum is varied. As stated above however, we found when we continued to increase the amount of anti-serum (the homologous serum being kept fixed) that the amount of precipitate formed might continue to increase, whilst the deviation of complement did not do so.

GENERAL CONSIDERATIONS.

The deviation of complement by a serum plus its anti-serum (presumably by a new compound formed) is one of the most striking of serum reactions, and opens up many questions of high theoretical importance. The relation of the phenomenon to precipitation has already been discussed at some length, and whilst there is a certain parallelism between them, we cannot say that it is the precipitate which fixes complement. The fact that deviation may occur where there is no precipitation would indicate that when a serum is injected the all-important result is the development of anti-substances, and these in combination with certain substances in the serum have the property of fixing complement. The combination of substance + anti-substance may be, and usually is, attended by precipitation. Further, the results with regard to the relation of the amount of complement fixed to the amount of precipitate, suggest that the latter may vary in composition. An analogy can be drawn between the anti-substances in question and the haemolytic and bacteriolytic immune-bodies. A striking difference, however, is presented by the fact that increase in the amount of serum (receptors) beyond a certain point leads to a diminution in the amount of complement taken up—a phenomenon which so far as we know has not been observed in the case of other anti-sera. The extraordinarily small amount of the homologous serum which is sufficient to produce a

recognisable deviation of complement also appears unique in serum reactions.

It has been recognised for a considerable time that when the serum of an animal is injected into another animal of different species, the serum of the latter acquires an "anti-complement" property. It will be evident however from what has been stated above that when the anti-serum is added to the serum, there are present the three essentials for the deviation phenomenon, viz. (*a*) certain molecules in the serum injected (homologues of receptors), (*b*) the anti-substances to these molecules, and (*c*) complement. Complement will thus be fixed and apparently neutralised. Are there in addition true anti-complements, *i.e.* anti-substances which unite directly with the haptophore group of complement in the same way as anti-toxin unites with toxin? The possibility of this cannot be excluded, but it is now clear that facts established with regard to anti-complement action are capable of another explanation. The question must be left an open one and requires fresh investigation in the light of the facts established with regard to deviation.

Summary of Results.

1. A mixture of serum and its anti-serum has the property of fixing or deviating complement and thus interfering with haemolysis. In this there is a close analogy to the fixation of complements by cell-receptors in association with immune-bodies.

2. A large number of different complements may be fixed by the same combination of serum and anti-serum: some complements however may not be fixed.

3. The amount of homologous serum necessary to produce a distinct deviation of complement is extremely small—·000,01 c.c. and even less: as a rule it is many times less than the amount necessary to give a visible precipitate with the anti-serum.

4. When a precipitate forms, the deviating substance is present in the precipitate and may be so exclusively: precipitation is however not essential, as the deviation phenomenon may be given by an anti-serum without the formation of a precipitate.

5. The precipitin and deviation tests give results which are in great part in accord as regards specificity.

6. For any given amount of anti-serum there is an optimum amount of homologous serum which gives maximum deviation of complement: above as well as below the optimum the deviation diminishes.

7. The deviation phenomenon produces an effect similar to an "anti-complement" action and the views generally held with regard to anti-complements require revision. It is however still left an open question whether true anti-complements exist.