ON THE RELATIONSHIPS BETWEEN THE COMPLEMENTS AND IMMUNE-BODIES OF DIFFERENT ANIMALS.¹

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IN this investigation I have endeavoured, by means of hæmolytic tests, to inquire into the phenomena observed when complements from different animals are used with different immune-bodies for the same corpuscles. The corpuscles used have been those of the ox, and immune-bodies for these have been obtained from the rabbit, cat, goat, duck, and ox² (isolysin); that is, these animals have been treated with successive injections of washed ox corpuscles, and after a suitable interval of time the blood has been drawn off; the serum is separated and heated at 55° C. to destroy complement, and the dosage of immune-body is then estimated in the usual way. The question thus comes to be as to how the complements of different animals behave with these immune-bodies.

There is comparatively little literature on the subject. Ehrlich and Morgenroth (1901¹), in their sixth communication on hæmolysis, compare the doses of immune-bodies of the rabbit and the goose for ox's corpuscles along with various complements, and from the table given by them it is seen, on the one hand, that pigeon's complement does not complement, or rather does not produce lysis, with the immune-body of the rabbit (a fact which I can confirm), whilst it lyses with the immune-body of the goose; and that, on the other hand, goat's complement produces lysis with rabbit's immune-body, but not with the goose's. Various examples of cross-complementing are, however, given, that is, examples where mammalian complements lyse well with avian immune-body and *vice versa*. They state, accordingly, that the general conclusion that avian complements in their entirety are different from those of mammals cannot be drawn, as is shown by the activation of rabbit's immune-body by the complements of the goose and chicken. Their observations were made in connection with the question as to the plurality of complements, and were interpreted on the basis of such plurality.

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Since the time of their publication I can find no systematic attempt to consider the matter. It is sometimes stated that avian complements do not act with mammalian immune-bodies, but this is quite incorrect, as, in fact, Ehrlich and Morgenroth themselves showed.

We may, however, in the first place consider what is meant by "complementing." The term *complement* was substituted by Ehrlich for his original one of *addiment*, to indicate that labile substance in a normal serum which produces lysis of corpuscles which have previously absorbed immune-body or have been "sensitised," and, as is well known, he believes that the complement combines with a special group in the immune-body (amboceptor) and is thus brought into relationship with the corpuscles. And, of course, the dose of complement is the smallest amount of normal serum which is sufficient to bring about complete lysis of the test amount of corpuscles (1 c.c. of a 5 per cent. suspension of the corpuscles in 0.8 per cent. sodium chloride solution) when these have been treated with the optimum amount of immune-body. When lysis occurs in such an experiment, we know, of course, that the complement acts with, or suits, the immune-body, and it can also be readily shown that the complement enters into firm union or is used up in the process of lysis; for the present, it is immaterial whether it combines with the immune-body or with the sensitised molecules of the red corpuscles. Accordingly, when lysis occurs a certain amount of complement has entered into combination, but beyond this a simple experiment, showing the dose of complement, teaches us little. Besides the phenomenon of chemical or physical combination of complements, there is also the factor of their toxic action, and this is found to vary much with the complements of different animals. Accordingly, variations in the dosage of different complements may be due to various causes, and these may be summarised as follows:—

(a) In the first place, the serum with relatively high dosage may have in its complement content comparatively few molecules which suit the immune-body in question, only a fraction will thus combine; this is Ehrlich's explanation in such cases, though it is doubtful whether it holds to any extent.

(b) In the second place, the complement molecules of two sera with different doses may combine equally well, but those of the one have a weaker toxic action than those of the other; and accordingly a much larger amount of the former is necessary in order to bring about lysis. In this case the minimum hæmolytic dose of the immune-body must also be larger in order to bring about combination of the more weakly acting complement with the corpuscles. Of this numerous examples might be given, but I may cite the case of ox's corpuscles sensitised with goat's immune-body along with guinea-pig's and sheep's complements. One might *a priori* expect that goat's immune-body would be better complemented by the latter, but so far as the mere occurrence of

lysis is concerned this is not the case, a much larger quantity of sheep's complement (normal serum) being necessary to produce lysis; it is also found that a much greater amount of immune-body (an amount about six times greater) is required than with guinea-pig's complement. The explanation in this and many other similar cases is that the ox's corpuscles are more sensitive to the action of guinea-pig's complement than of sheep's complement, and that, accordingly, a larger amount of immune-body is necessary to bring into union the more weakly acting complement in amount sufficient for lysis. The result manifestly gives no support to the view that guinea-pig's complement suits the immune-body of the goat better than does sheep's complement. There are also instances where a normal serum does not produce lysis of sensitised corpuscles and yet union of complement, *i.e.* "complementing," occurs. For example, ox's corpuscles sensitised by the immune-body of the rabbit are not lysed by horse's complement, yet it is found by the deviation or subtraction method that a considerable amount of the latter has entered into combination; in other words, *complementing without lysis* has occurred. Ox's corpuscles are not lysed by ox's complement with any of the immune-bodies tested, though a considerable amount of complement becomes united to the sensitised corpuscles. This will be referred to below. (c) In the third place, the relatively high dose of one "complement" (normal serum) as compared with another may be due to the fact that the former contains a smaller number of complement molecules,—the complement is less concentrated. For example, it was shown by Browning and myself (1904²) by means of substitution experiments, that the relatively high dose of rabbit's as compared with guinea-pig's complement for ox's corpuscles sensitised with rabbit's immune-body, was in great part due to this cause.

It is thus manifest that when a complement does not lyse sensitised corpuscles, or does so only in comparatively large doses, we can say nothing in explanation from a simple hæmolytic experiment—we cannot say without further analysis whether it complements badly or not so far as combination is concerned. The only proper measure of complementing, in Ehrlich's sense, is given by the amount of complement which is fixed by a given amount of sensitised corpuscles according to the Bordet-Gengou principle. Some years ago I showed (1903³) by such a method that corpuscles which had absorbed multiple doses of immune-body might fix several doses of complement, in fact, in one instance the amount of the latter was approximately proportional to the amount of immune-body. Theoretically this principle should give clear information as to complementing, and I have applied it in a considerable number of instances. Unfortunately, however, all complements do not behave in the manner indicated. For example, if we take the case of cat's complement along with ox's corpuscles sensitised with cat's immune-body, we find that not much

more complement is fixed by ten doses than by a single dose of immune-body; while, on the other hand, the amount of guinea-pig's complement fixed is nearly proportional to the amount of immune-body. In other words, guinea-pig's complement combines better, *i.e.* in larger amounts, through the medium of cat's immune-body than does cat's complement. There is, of course, the possibility that the union of cat's complement is comparatively weak, and that a certain amount dissociates again. In any case, in view of the fact stated, it is not surprising that cat's complement does not combine in proportional amounts with other immune-bodies, and accordingly a comparison of its properties cannot be made by this method. Nevertheless, the method gives important information in certain instances and the results obtained by it will be found below.

I shall, in the first place, give in tabular form the hæmolytic doses of the complements of different animals for ox's corpuscles sensitised by the five immune-bodies used. The activity of different samples of complement of the same species of animal was found to vary considerably, but the figures stated may be taken as indicating a fair average; the estimation of the dose of each complement was made for all the immune-bodies at the same time. In cases where the fresh normal serum had a hæmolytic action on the ox's corpuscles, the natural immune-body was as far as possible removed by placing the serum in contact with the ox's corpuscles for an hour at 0° C. The sign ∞ used in relation to the dose of a complement is not to be interpreted in a strict manner, but merely as meaning that the complement (fresh serum) in question has in a dose of 1 c.c. little or no hæmolytic action on the test amount of corpuscles sensitised with the immune-body stated.

Complement of	Doses of Complement (in Cubic Centrimetres) with the Immune-bodies of—				
	Rabbit.	Goat.	Cat.	Ox.	Duck.
Guinea-pig . . .	0·01	0·013	0·03	0·015	∞
Rabbit	0·1	0·15	0·14	0·1	0·1
Goat	0·4	0·1	0·4	0·2	∞
Sheep	0·85	0·2	0·25	0·8	∞
Ox	∞	∞	∞	∞	∞
Horse	∞	∞	0·2	∞	∞
Pig	0·11	0·15	0·12	0·1	0·15
Man	0·12	0·12	0·12	0·15	0·6
Dog	0·2	0·2	0·4	0·5	1·0
Cat	0·3	0·3	0·2	{ almost ∞ }	0·2
Duck	0·25	0·3	0·1	0·3	0·075
Fowl	0·27	0·3	0·3	0·7	0·35
Pigeon	∞	∞	∞	∞	0·05

On examining the above table, it will be seen that, on the whole, mammalian complements lyse better with the mammalian immune-

bodies than do avian complements, and that the avian complements lyse better with the avian immune-body than do mammalian complements, although to these statements there are many exceptions. Thus, of the ten mammalian complements used five produce practically no lysis with the duck's immune-body; but of these one, namely, the ox's complement, does not lyse with any of the four mammalian immune-bodies, whilst the horse's complement does not lyse with three of them, and the dosage of two other complements, namely, the goat's and the sheep's, even with mammalian immune-bodies, is very high. The non-production of lysis by mammalian complements along with avian immune-body is therefore not so marked as at first it seems to be. The most striking example is seen in the case of the guinea-pig's complement, which produces lysis in very small doses with all the mammalian immune-bodies, and, on the other hand, does not lyse with the duck's immune-body. It can also be shown that in the latter case the guinea-pig's complement is not taken up, that is, does not enter into combination. Of the others, the complements of the rabbit, cat and pig lyse in as small doses with the duck's as with the mammalian immune-bodies, whilst the dose of the complements of man and the dog are higher with the avian immune-body. The balance is thus distinctly in favour of more efficient lysis by mammalian complements with mammalian immune-bodies than with avian immune-body, but more than this cannot be said.

When we come to the avian complements we find considerable variations in their behaviour. The most noteworthy case is that of the pigeon's complement, which fails to lyse with the mammalian complements but lyses in comparatively small doses with the duck's complement. In fact, if the results obtained by using guinea-pig's and pigeon's complements alone had been considered, it would have appeared that there was no cross-complementing as regards mammalian and avian immune-bodies, the former complement lysing only with mammalian immune-bodies and the latter only with the avian immune-body. As a contrast, however, to the case of the pigeon's complement, it will be seen that the fowl's complement lyses well with all the mammalian immune-bodies, and in the case of three of them in as small a dose as with the duck's immune-body; so also the duck's complement lyses well with all the mammalian immune-bodies, and its dose with the cat's immune-body is only a trifle higher than with the immune-body of the duck itself.

It is thus evident that, when all the facts are considered, it is impossible to dispose the co-working of complements and immune-bodies according to zoological groupings. The production of lysis by guinea-pig's complement with all the mammalian immune-bodies, and the absence of lysis with the avian immune-body, on the one hand, and the production of lysis by pigeon's complement along with avian immune-body and the absence of lysis with the mammalian immune-

bodies, on the other hand, are no doubt striking facts, but they are exceptional. Why, for example, should the fowl's and the duck's complement behave in a way so markedly different from the pigeon's? And how can one explain the circumstance that pig's complement lyses almost equally well with all five immune-bodies—four mammalian and one avian? It will also be noted that the cat's complement behaves in a somewhat similar manner to the pig's and lyses well with different immune-bodies in approximately the same dose. Such facts cannot be explained according to the theory of a complementophile group in the immune-body with specific character, as it is manifest that such a group is present in immune-bodies of widely different origin. Rather must we suppose that there is some relatively simple condition, physical or chemical, brought about by immune-body, which determines whether combination of complement, and also lysis, takes place or not.

We have stated that guinea-pig's complement does not lyse with duck's immune-body, and is not taken up by means of it; but Ehrlich and Morgenroth¹ found that the guinea-pig's complement lyses well with goose's immune-body. In fact, the dose of the latter with guinea-pig's complement is the lowest recorded in the table. It is impossible at present to explain why immune-bodies from two so closely allied animals as the duck and goose should behave so differently with the same complement. An even greater difficulty, as regards interpretation, is brought out in the case of rabbit's and guinea-pig's complements. For example, it has been shown by Brown-ing and myself² that guinea-pig's and rabbit's complement have apparently the same combining group when tested on ox's corpuscles sensitised with the immune-body of the rabbit—each excludes the other from combination. In other words, the combining groups of the rabbit's and guinea-pig's complements appear to be identical. It is also to be noted that the dose of immune-body (rabbit *v.* ox) with these two complements is practically identical; that is, it is practically immaterial whether a combined hæmolytic dose of immune-body is saturated by guinea-pig's or rabbit's complement, so far as lysis is concerned. If, however, we take the case of the immune-body of the duck for ox's corpuscles we find that the rabbit's complement combines readily and produces lysis in a comparatively small dose, in fact, in as small a dose with the duck's immune-body as with the mammalian immune-bodies; on the other hand, the guinea-pig's complement dose not lyse the sensitised corpuscles at all and does not even combine with them. Accordingly, in this latter case, the supposed combining groups of the rabbit's and the guinea-pig's complement behave as if they were quite different. We thus see that when two complements behave in a similar way as regards combination with one immune-body, it does not at all follow that

¹ *Loc. cit.*

² *Loc. cit.*

this will hold in the case of another immune-body. The facts stated show clearly that with slight zoological differences as regards the sources of immune-bodies and complements, great differences as regards the occurrence or non-occurrence of lysis are brought out. In fact, the differences in the behaviour of the immune-bodies of the goose and of the duck with guinea-pig's complement, on the one hand, and in the behaviour of rabbit's and guinea-pig's complements with the immune-body of the duck, on the other hand, are as great as can be met with. The combining affinities of complements have thus no definite relationship to zoological groups.

Having thus considered the action of complements as regards the production of lysis and their dosage with different immune-bodies, we have next to inquire further into the question of their combining affinities. This may be conveniently done by examining the cases where lysis does not occur and thereafter those in which it does occur.

1. LYSIS DOES NOT OCCUR.—Theoretically the non-occurrence of lysis may be due either to the non-union of complement (the complement does not "complement") or to the corpuscles being non-sensitive to the toxic action of complement after it has combined. Both of these conditions are found to obtain.

(a) *The complement does not combine.*—This is found to be the case with guinea-pig's complement in relation to ox's corpuscles sensitised with the immune-body of the duck. This may be shown by the following type of experiment:—

Two series of test tubes are taken, each containing 1 c.c. of the suspension of corpuscles. In the one series (a), the corpuscles are fully sensitised with several doses of duck's immune-body, and, after time is allowed for combination, the fluid is removed by centrifugalising and is replaced by fresh salt solution; the suspension of corpuscles in the tubes of series (b) is left untreated. To the tubes of each series we add increasing amounts of guinea-pig's complement, beginning with a little less than a single dose, and the tubes are then placed in the incubator for an hour 37° C. After that time the fluid from each tube is separated by means of the centrifuge and added to 1 c.c. of corpuscles sensitised with rabbit's immune-body; the resulting lysis, of course, shows the amount of complement which has remained uncombined. The experiments carried out showed that the amount of lysis was practically the same in the two series; in other words, that practically no complement was taken up by means of the duck's immune-body.

(b) *The complement combines through the medium of the immune-body, but lysis does not occur.*—This is merely the extreme example of variation in the toxicity of complements towards different corpuscles. Such variations were first shown by Browning and myself (1904⁴) in the case of rabbit's and guinea-pig's complements towards the homologous sensitised corpuscles; the doses of these complements being in each case very high, owing to the relative non-sensitiveness of the corpuscles of an animal to its own complement. This factor of varying toxicity was also recognised by Bordet and Gay (1906⁵). Other examples of a similar nature might be mentioned. Ox's complement, for

example, is taken up, although not to a great extent, by means of all the four mammalian immune-bodies used without the corpuscles being lysed as a result; in other words, ox's complement has little hæmolytic effect on sensitised ox's corpuscles, even when it combines with them. Accordingly, when no lysis occurs in a hæmolytic experiment, we cannot say, without further investigation, whether the complement has entered into combination or not with the sensitised corpuscles.

2. LYSIS OCCURS.—In such cases the amount of complement taken up in proportion to the amount of immune-body has to be studied by the Bordet-Gengou method. Some years ago¹ I showed that guinea-pig's complement is fixed or deviated by ox's corpuscles sensitised by rabbit's immune-body in amounts roughly proportional to the amount of immune-body used. Similar experiments carried out in the present research have given corresponding results as to guinea-pig's complement acting along with the immune-bodies of the ox, goat, and cat; with all of these, many times the amount of complement necessary for lysis may be fixed. As already mentioned, the cat's complement behaves in a different way. Even along with cat's immune-body, scarcely more complement is fixed by ten doses of immune-body than by one dose, and a similar result is obtained when the other immune-bodies are employed. These two complements may be taken as the extreme examples as regards the amount of deviation in proportion to the amount of immune-body, most of the other complements occupying an intermediate position. Thus rabbit's complement is deviated well, although not quite in such high proportions as guinea-pig's complement; it is, however, fixed quite as well through the medium of goat's immune-body as rabbit's immune-body. Here again there is no evidence of a specific combining group in the rabbit's complement. It is hardly possible to suppose that such a group would be present in a similar manner in goat's immune-body. As regards other complements the following results may be mentioned. Human complement, sheep's complement, and pig's complement are deviated fairly well by the immune-bodies of the rabbit and goat; in some experiments sheep's complement was fixed to an equal degree by these two immune-bodies, and in others again rather better by the goat's. The only clear example of better fixation (deviation) of homologous complement is given in the case of a goat's complement along with goat's immune-body as compared with rabbit's immune-body, distinctly more complement being taken up through the medium of the former. A small amount of ox's complement is taken up by the four mammalian immune-bodies used, but not nearly in proportion to the amounts of these, *i.e.*, it is a complement which is not deviated well. These facts accordingly show that the complements of different animals vary greatly as

¹ *Op. cit.*

regards the amount of combination in proportion to the amount of immune-body, even when the homologous immune-body is used.

THE MODE OF ACTION OF IMMUNE-BODY.

Another question, to which hitherto little or no attention has been paid by writers on the subject, is whether or not the immune-body plays any other part in the phenomenon of lysis than that of bringing the complement into relation with the molecules of the red corpuscles. We know, of course, that an immune-body has no lytic action of itself, but it does not necessarily follow that the effect of a given complement will be the same with immune-body A as with immune-body B. If there is a difference, it will have to be shown that (1) all the complement has combined in the two cases, and (2) that the immune-bodies combine with the same molecules of the red corpuscles. That these conditions actually obtain is shown by the following examples.

I may first quote the case of sheep's complement acting along with rabbit's and goat's immune-bodies, the dose of the complement with the former immune-body being much the higher. An experiment to test the amount of sheep's complement taken up is made as follows:—

The dose of sheep's complement with the two immune-bodies was found to be, in one case, 0.2 c.c. with goat's immune-body and 0.55 c.c. with rabbit's. Two series of tubes are taken, each tube containing 0.5 c.c. of the suspension of ox's corpuscles. The corpuscles in one series are saturated with rabbit's immune-body and in the other with goat's immune-body. The surplus fluid, after time is allowed for combination of immune-body, is separated by the centrifuge and the corpuscles are re-suspended in salt solution. We then add to the tubes in each series varying amounts of sheep's complement, 0.05, 0.1, 0.2 c.c., etc., and the tubes are incubated for one and a half hours at 37° C. We then test for the presence of free complement by adding 0.1 c.c. of suspension of ox's corpuscles sensitised with goat's immune-body. This will show the presence of 0.02 c.c. of free complement (in the case of the tubes where initial lysis is incomplete, the corpuscles are separated by means of the centrifuge and the fluid is used to test for free complement). The result obtained was that *in both series* the tube to which 0.25 c.c. of complement had been added originally showed imperfect lysis of the test corpuscles added; in other words, the amount of complement combined in the two series is the same, though the amount of original lysis was much greater in the case of the tubes sensitised with goat's immune-body than with rabbit's.

It is thus seen that the high dose of sheep's complement with rabbit's immune-body as compared with the dose with the goat's, is not due to only a fraction of the complement combining in the former case; the whole of the complement lytic for the corpuscles in question has combined. Accordingly, we may say that 0.2 c.c. of sheep's complement combined through the medium of goat's immune-body produces complete lysis of 1 c.c. of the test corpuscles, whilst the same amount combined by means of rabbit's immune-body produces little lysis; this difference must depend in some way on the immune-body. It should be added

that Poggenpohl (1909⁶) found that the immune-body of the goat combined with the same molecules of the red corpuscles of the ox as did the immune-body of the rabbit,—corpuscles saturated with the one immune-body are saturated for the other,—and I have obtained a similar result. The difference in the amount of lysis therefore cannot depend upon different receptors of the red corpuscles being concerned.

Another closely similar example is afforded by the action of guinea-pig's complement along with rabbit's and cat's immune-bodies, its dose with the latter being three times that with the former, and it may be added that lysis occurs more quickly with the rabbit's immune-body than with the cat's. Here again it can be shown that there is no failure in the combination of complement with cat's immune-body to explain the difference. When, for example, 0.02 c.c. of complement (twice the hæmolytic dose with rabbit's immune-body) has been allowed to act for a suitable time on corpuscles fully sensitised with cat's immune-body, all the complement has entered into combination, yet lysis is incomplete. I have also found by saturation experiments that the cat's and rabbit's immune-bodies combine with the same molecules of the red corpuscles, each excluding the other from combination.

Perhaps the most striking example of all is found in the case of horse's complement acting with rabbit's and cat's immune-bodies. With the former immune-body no lysis occurs, but, as Browning (1906⁷) and also Bordet and Gay¹ have shown, a considerable amount of complement is fixed. I have found, for example, in one experiment that 0.5 c.c. of horse's complement was fixed by the test amount of ox's corpuscles sensitised with rabbit's immune-body without there being any lysis, *i.e.*, fully twice the amount of complement which would have produced complete lysis of the corpuscles had they been sensitised with cat's immune-body. (In such experiments I have used as the test for free complement either (*a*) guinea-pig's corpuscles sensitised with the natural immune-body of the ox, or (*b*) ox's corpuscles sensitised with cat's immune-body, the sensitised corpuscles in each case undergoing lysis when free horse's complement is present). There is thus a great difference so far as lysis is concerned according as to whether the corpuscles are sensitised with rabbit's or with cat's immune-body, though the complement combines in both cases and the two immune-bodies can be shown to unite with the same molecules of the red corpuscles.

These results accordingly show that it is not immaterial by what immune-body the complement is brought into union with the corpuscles. The fact that certain complements, *e.g.* that of the pig, produce lysis in approximately the same doses with the different immune-bodies tested, would indicate that in these instances the lysis is little affected by the immune-body used; but the examples quoted above show that this is not always the case, and indicate clearly that the immune-body

¹ *Op. cit.*

in certain cases may play a part in the lytic process though we cannot at present say how it acts. I have considered above the various factors which affect the hæmolytic doses of complements, and to these the nature or source of the immune-body must now be added as another factor, the immune-body not merely regulating the combination of complement (this is already well recognised), but also influencing the resulting lysis.

SUMMARY OF RESULTS.

1. There is a balance in favour of more effective complementing of mammalian immune-bodies by mammalian complements than by avian complements, and *vice versa*.

2. On the other hand, slight zoological differences (*e.g.* as between rabbit and guinea-pig or between duck and goose) may be associated with the most marked divergence in the mode of action of complements or immune-bodies.

3. The facts obtained are not in accordance with the idea of there being special complementophile groups in immune-bodies.

4. When lysis of sensitised corpuscles does not occur on the addition of a complement, this may be due either to (*a*) non-combination of the complement (*i.e.* "non-complementing") or to (*b*) non-sensitiveness of the corpuscles to the action of complement, which has nevertheless really combined.

5. In cases where lysis of sensitised corpuscles occurs, the quantity of complement fixed may be roughly in proportion to the amount of immune-body, *i.e.*, it is deviated well. On the other hand, this may not be the case, multiple doses of immune-body fixing little more complement than a single dose. The result depends more on the complement used than on the sensitised corpuscles.

6. The result in a hæmolytic experiment depends chiefly on the complement and the corpuscles used. It is, however, not immaterial, as regards the amount of lysis, by what immune-body the complement is brought into combination with the corpuscles. More efficient lysis may result with one immune-body than with another, the same complement being used in the two cases, and this even when it can be shown that the immune-bodies unite with the same molecules (receptors) of the corpuscles. These facts are best explained on the assumption that the immune-body, although not producing lysis by itself, acts as an auxiliary toxic agent in addition to leading to the union of complement.

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