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BACTERIOLYTIC POWER OF IMMUNE SERUM AND THE THEORY OF COMPLEMENT DIVERSION.[1]

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In a previous paper it was shown that one cubic centimeter of normal rabbit serum might be expected to kill — of

Typhoid bacilli, about . . .	1,000,000
Paratyphoid bacilli, about . .	50,000,000
Cholera bacilli, about . . .	100,000,000

and in the present communication the influence of immunization of rabbits to these organisms upon the bactericidal power of their serum will be considered. Rabbit serum only was used in the experiments.

The most interesting points which have developed during the course of the work related to the so-called complement diversion (Komplement Ablenkung) of Neisser and Wechsberg, and a discussion of this phenomenon will form the subject matter of the article.

Diagrams have been freely used, for they enable a rapid mental picture to be formed which makes the description much easier to follow.

Passive Immunization. — It is well known that immune serum, inactivated at 56° C. and injected into an animal, together with a dose of bacilli considerably above the minimum lethal dose, will protect against such a dose by a process

[1] Received for publication April 14, 1905.

of passive immunization, there being naturally a minimum dose of immune serum below which there is no protection.

In 1896, however, Löffler and Abel[1] observed that, on injecting typhoid immune serum and typhoid bacilli into guinea-pigs, not only is there a minimum dose below which there is no protection, but there is also a maximum dose of serum above which it does not protect the animal. It is only moderate doses of immune serum which afford protection from death by typhoid bacillemia.

These experiments can be repeated and confirmed as appears in the following abbreviated table taken from among my own experiments with paratyphoid. Guinea-pigs of about two hundred and fifty grams weight were used, the dose, about 3 M. L. D., being one-eighth of a living agar culture per one hundred grams weight of pig.

TABLE I.

Paratyphoid bacilli and paratyphoid immune serum.

Guinea-pig.	Dose per 100 grams.	Immune Serum.	Result.	
1.....	1/8th culture.	0	Died.	Control.
2.....	" "	1 cc.	"	Pro-zone. The bacilli multiply.
3.....	" "	.1 cc.	Lived.	Killing-zone. The bacilli are killed.
4.....	" "	.01 cc.	Died.	Post-zone. The bacilli multiply.

For some time after Löffler and Abel's observations no explanation was forthcoming for this singular phenomenon, until, in 1901, Neisser and Wechsberg[2] made a series of experiments "in vitro" with analogous results. On treating typhoid bacilli with inactivated (at 56° C.) immune serum plus fresh complement-containing normal serum, only medium doses of the immune serum would kill the bacilli. Both above and below this killing-zone the bacilli were not affected and grew freely in the fluid. One can readily

[1] Centralblatt für Bakteriologie, Vol. 19, 1896, p. 51.

[2] Münchener medizinische Wochenschrift, No. 18, 1901.

repeat these experiments and satisfy himself that the observations of Neisser and Wechsberg are correct. The accompanying tables for typhoid and paratyphoid are selected from my own tests.

The tubes were made up as follows:

1. The immune serum, inactivated at 56° C., was diluted to the various strengths required with salt solution and one cubic centimeter of the dilution pipetted into each tube.

2. The fresh complement-containing normal serum was diluted (two cubic centimeters to eight cubic centimeters salt solution) and one cubic centimeter pipetted into each tube (= .2 cc. in each tube).

3. Three drops of broth were added to each tube to afford a medium for growth in cases where the bacilli were not killed. Neisser and Wechsberg recommend this procedure.

4. Five drops of a very dilute emulsion of bacilli were also added to each tube.

For plating, five drops of the fluid were taken. Each tube, therefore, contained two cubic centimeters, together with three drops of broth and five drops of bacillary emulsion. Agar plates were made from each tube at once and in five hours, the fluid being again examined for growth at the end of twenty-four hours.

TABLE II.

Inactivated typhoid immune serum and typhoid bacilli.

	Immune Serum.	Normal Serum.	Colonies at Once.	Colonies in 5 Hours.	Fluid in 24 Hours.	
1.	1 cc.	0	Many thousands.	Very many thousands.	Growth	Controls.
2.	0	.2 cc.	" "	Many thousands.	Growth	Controls.
3.	1 cc.	.2 cc.	" "	" "	Growth	Pro-zone.
4.	.3 cc.	.2 cc.	" "	Few thousands.	Clear	Killing-zone.
5.	.1 cc.	.2 cc.	" "	" "	Clear	Killing-zone.
6.	.03 cc.	.2 cc.	" "	" "	Clear	Killing-zone.
7.	.01 cc.	.2 cc.	" "	Many thousands.	Growth	Post-zone.
8.	.003 cc.	.2 cc.	" "	" "	Growth	Post-zone.
9.	.001 cc.	.2 cc.	" "	" "	Growth	Post-zone.

TABLE III.

Inactivated paratyphoid immune serum and paratyphoid bacilli.

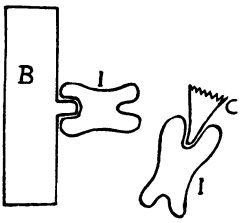
	Immune Serum.	Normal Serum.	Colonies at Once.	Colonies in 5 Hours.	Fluid in 24 Hours.	
1.	1 cc.	0	Many thousands.	Very many thousands.	Growth	Controls.
2.	0	.2 cc.	" "	Many thousands.	Growth	Controls.
3.	1 cc.	.2 cc.	" "	Very many thousands.	Growth	Pro-zone.
4.	.3 cc.	.2 cc.	" "	" " "	Growth	Pro-zone.
5.	.1 cc.	.2 cc.	" "	Thousands.	Clear	Killing-zone.
6.	.03 cc.	.2 cc.	" "	100	Clear	Killing-zone.
7.	.01 cc.	.2 cc.	" "	100	Clear	Killing-zone.
8.	.003 cc.	.2 cc.	" "	100	Clear	Killing-zone.
9.	.001 cc.	.2 cc.	" "	200	Clear	Killing-zone.
						Post-zone.

The tables show that the killing-zone for the typhoid immune serum is from .03 cubic centimeter to .3 cubic centimeter inclusive. Both above and below this (pro- and post-zones) the bacilli are not killed.

With the paratyphoid immune serum the minimum killing dose of immune serum has not in this instance been reached, although at .001 cubic centimeter the effect is lessened. Above .1 cubic centimeter, however, the bacilli are not affected.

Neisser and Wechsberg explained this phenomenon by supposing that the complements and immune bodies must combine together before the latter become attached to the bacilli, forming a complement-immune body combination. If now the immune bodies are present in excess there will be many left free even after absorption of all the complements, and many of the bacilli will become attached to free immune bodies to the exclusion of the complement-immune body combinations. Such bacilli remain unaffected and are able to multiply freely in the serum.

DIAGRAM I. — *Diversion of Complements.*



B. Bacillus.
I. Immune bodies.
C. Complement.

The bacillus is occupied by an immune body, but the complement necessary for its destruction is diverted by a free immune body.

Whether the experiment is made "in vitro" or with a normal animal the result is the same. An excess of immune serum permits the growth of the bacilli.

This is Neisser and Wechsberg's "diversion of complements," a theory which has been very generally accepted and is usually referred to as being the only possible explanation of the phenomenon.

Bordet, however, has raised some objections which will be referred to later on. For the present we may accept the theory as accounting for the phenomena described and see if it will stand other tests.

Let us first consider the action of fresh immune serum on its specific bacillus as compared with that of fresh normal serum on the same bacillus. In this case the immune serum contains its own complements instead of extraneous ones, as in the Neisser and Wechsberg experiments. In Tables IV. and V. the averages of a large number of experiments are taken. To one-half cubic centimeter of serum loopfuls or drops of bacillary emulsions are added, and the number of bacilli per cubic millimeter estimated from the colonies on the plates. Details of the methods employed were given in the previous paper on normal serum already referred to.

TABLE IV.

Serum ½ cc.	Typhoid Bacilli per Cubic Millimeter of Serum.			Serum in 24 Hours.	Averages of
	Number of Bacilli Inoculated.	In 2 Hours.	In 5 Hours.		
Normal.....	1,170	103	4	Usually sterile. Sometimes growth.	20 tests.
Immune to typhoid, 7 rabbits	620	630	2,250	Always growth.	20 tests.

TABLE V.

Serum ½ cc.	Paratyphoid Bacilli per Cubic Millimeter of Serum.			Serum in 24 Hours.	Averages of
	Number of Bacilli Inoculated.	In 2 Hours.	In 5 Hours.		
Normal	32,000	22	0	Always sterile.	11 tests.
Immune to paratyphoid, 3 rabbits.	3,000	2,000	10,000	Always growth.	7 tests.

NOTE. — It will be observed that the average number of bacilli per cubic millimeter inoculated into the immune serum is considerably below the number inoculated into the normal serum. The reason for this is that finding, contrary to the first expectations, that the immune serum was very slightly, if at all, bactericidal, the quantity of bacilli inoculated was constantly cut down, thus lowering the averages.

It may also be remarked that a number of rabbits, in addition to those tabulated, were immunized to the bacilli in question, but since their serums were tested by a somewhat different method, the experiments cannot be included in these averages. The net results, however, were always the same. The immune serums are not bactericidal except under conditions mentioned later on.

The tables show that the serums immune to typhoid and paratyphoid, fresh, and therefore containing their own complement, do not kill any of their own specific bacilli at all, whereas normal rabbit serum is always highly bactericidal.

This must be due to the same causes as the previously

mentioned phenomena, and for the present we may regard it as a case of diversion of complements, owing to excess of immune bodies, and picture the condition as corresponding to that illustrated in Diagram I.

Cholera immune serum, however, has shown no such phenomenon with the two rabbits so far tested.

TABLE VI.

Serum ½ cc. 18 Hours Old.	Cholera Bacilli per Cubic Millimeter of Serum.			Serum in 24 Hours.	Averages of
	Number of Bacilli Inoculated.	In 2 Hours.	In 5 Hours.		
Normal.....	50,000	0	0	Always sterile.	20 tests.
Immune to cholera, 2 rabbits.....	42,000	0	0	Always sterile.	4 tests.

Pfeiffer [1] has never observed diversion of complements "in vivo" on passively immunizing animals with cholera immune serum.

Let us now consider the effect upon cholera bacilli of heterologous immune serums.

TABLE VII.

Serum ½ cc. 18 Hours Old.	Cholera Bacilli per Cubic Millimeter of Serum.			Serum in 24 Hours.	Averages of
	Number of Bacilli Inoculated.	In 2 Hours.	In 5 Hours.		
Normal.....	50,000	0	0	Always sterile.	20 tests.
Immune to typhoid.	52,000	0	0	Always sterile.	14 tests.
Immune to paratyphoid.....	85,000	8	0	Always sterile.	7 tests.

[1] Centralblatt für Bakteriologie, Vol. 35, Ref. 1904, p. 244.

Serums immune to typhoid and paratyphoid will kill cholera to just about the same extent as normal serum. The complements for cholera have not been diverted, although, as we have seen, they are diverted for typhoid and paratyphoid respectively. But complements are usually considered to be general, not specific, although some experiments point to the probability of certain complements being special.

Accepting diversion of complements for typhoid and paratyphoid, we must conclude from these experiments on cholera bacilli with heterologous serums either:

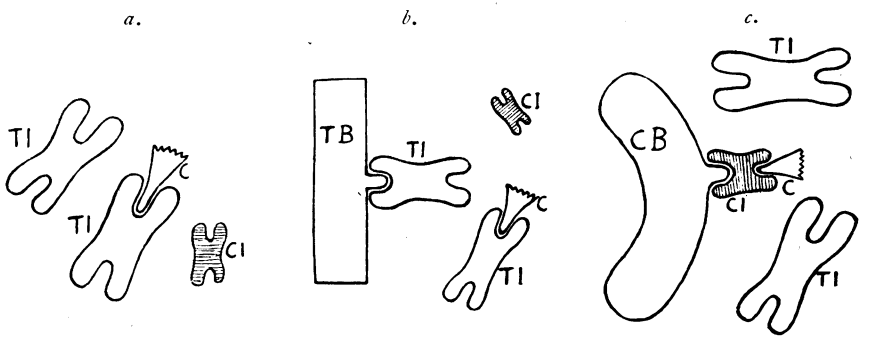
1. That the complements for cholera are entirely different from those for typhoid and paratyphoid, or

2. That the mere presence of typhoid bacilli (paratyphoid) in the immune serum so influences the complements as to cause them to combine with the free immune bodies without the bacilli themselves entering into the reaction at all, while introduction of cholera bacilli into typhoid immune serum produces no such effect. In the former case, on adding typhoid bacilli, there is a complement-immune body combination formed, but not in the latter case on adding cholera bacilli, or

3. That on addition of cholera bacilli to typhoid immune serum the affinities of the complements are so altered that they leave the typhoid immune bodies to attach themselves to the cholera intermediate bodies.[1] Diagram II. explains the hypothesis.

[1] The amboceptors of normal serum are here called "intermediate bodies" (Zwischenkörper of Ehrlich), as distinguished from the "immune bodies" (Immunkörper) of immune serum.

DIAGRAM II.



TI Typhoid immune body.
CI Cholera intermediate body.
C Complement.
CB Cholera bacillus.
TB Typhoid bacillus.

DIAGRAM II., *a*. — Shows the conditions in typhoid immune serum according to the hypothesis of complement diversion. An excess of immune bodies over complements, the latter occupying some of the immune bodies. There are also free cholera intermediate bodies.

DIAGRAM II., *b*. — Represents the conditions on introducing typhoid bacilli. The free immune body has attached itself to the bacillus to the exclusion of the complement-immune body combination. The bacillus therefore escapes destruction.

DIAGRAM II., *c*. -- Typhoid immune serum to which cholera bacilli are added. The cholera intermediate body attaches itself to the cholera bacillus, and the complement leaves the typhoid immune body to attach itself to the cholera intermediate body. The cholera bacillus is therefore killed.

Of these three hypotheses, one of which we must accept in order to maintain the theory of complement diversion, the two latter entail such forced assumptions that they are hardly worthy of consideration, and the first, namely, that complements are specific, is certainly opposed to many facts which have been observed.

For instance, if fresh normal serum is allowed to kill a maximum dose of cholera bacilli (about 100,000,000 per

cubic centimeter of serum) and then treated with typhoid bacilli, it will no longer kill any of the latter, whereas control normal serum kept under similar conditions, but without the addition of cholera bacilli, will kill about a million typhoid bacilli per cubic centimeter.

In this case, as was shown in the previous article referred to, it seems certain that the cholera bacilli have absorbed the complements for typhoid to such an extent that there are no longer enough left to kill typhoid bacilli. This being so we must conclude either:

(1.) That cholera can absorb not only its own special complements, but also the special complements for typhoid, or

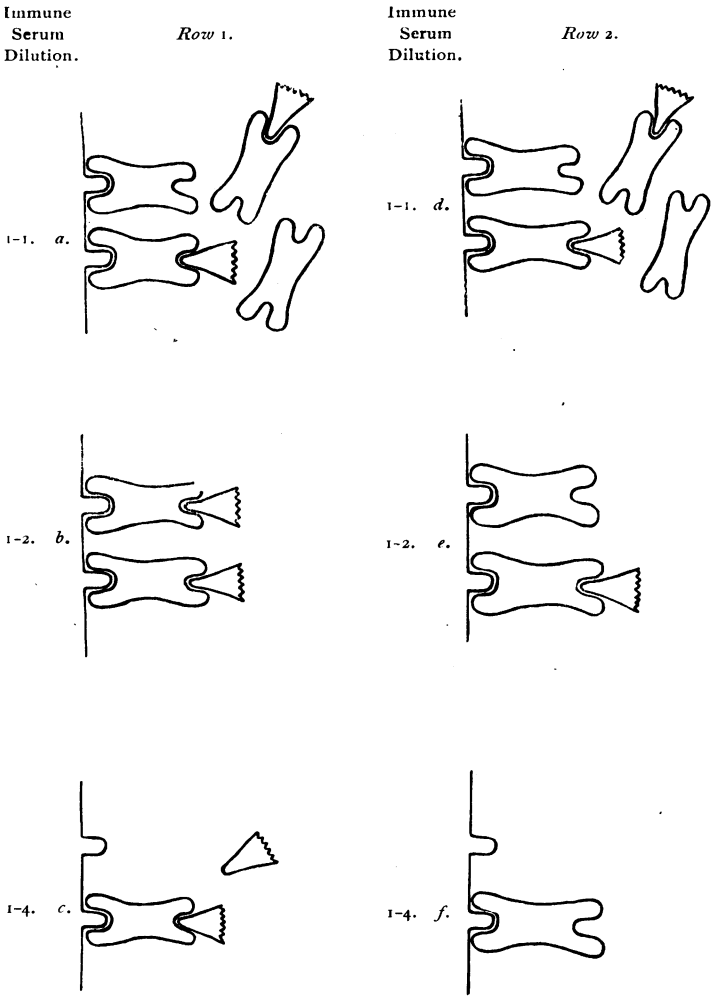
(2.) That complements are general for both.

The latter is the more reasonable conclusion.

Our faith in the hypothesis, or complement diversion, having been somewhat shaken, we can put the theory to another test.

Supposing fresh immune serum is diluted. In this case we are diluting both the immune bodies and complements together, and if complements are diverted in undiluted serum, there is no reason to suppose that they are not also diverted in dilute serum, so that dilute fresh immune serum should not be any more bacteriolytic than undiluted.

DIAGRAM III.



In Diagram III. a typhoid bacillus with two receptors is figured on the left of each division, and we may suppose that both receptors must be occupied by a complement-immune body combination in order to kill the bacillus.

We may also suppose the immune serum to be diluted 1–1 (*a* and *d*), 1–2 (*b* and *e*), and 1–4 (*c* and *f*).

Row 1. — *a*, *b*, and *c* represent the condition in the Neisser and Wechsberg experiments with a fixed amount of complement, and Row 2, *a*, *e*, and *f* represent fresh immune serum containing its own complement.

In Row 1 the amount of complement added to the inactivated immune serum is a constant. In this supposititious case there are two complements at each dilution.

In *a* (Dilution 1–1) there are four immune bodies, one of which diverts the second complement from the bacillus so that it is not killed.

In *b* (Dilution 1–2) the number of immune bodies and complements is the same, so both the complements are utilized and the bacillus is killed.

In *c* (Dilution 1–4) the immune bodies are too few in number, so the second complement remains free and the bacillus escapes.

We here have, then, the pro-, killing-, and post-zones in miniature.

Taking Row 2, *d*, we may suppose that in the fresh immune serum there are two complements and four immune bodies, and the conditions are similar to that depicted in Row 1, *a*. There is a diversion of one complement, and the bacillus escapes destruction. On diluting the serum to 1–2, as in *e*, there are now two immune bodies and only one complement. The bacillus, therefore, should survive.

In *f* (Dilution 1–4) there is only one immune body and may or may not be one complement. In any event, however, there would not be sufficient either of immune bodies or complements to destroy the bacillus.

Accepting the theory of complement diversion as explaining the phenomenon in concentrated immune serum, whether fresh or inactivated, it is obvious that the fresh immune serum should not be able to destroy the bacilli at any dilution, contrary to what occurs if the amount of complement is a constant.

Tables VIII. and IX. give two experiments out of a large

number made to test this point, all the tests affording very similar results. Normal serum was also tested for comparison.

TABLE VIII.

Serums. — Normal and immune to typhoid. Bacilli typhoid. — Five drops of thin emulsion in each tube. Each tube made up to two cubic centimeters and three drops of broth added.

	Serum 18 Hours Old Dilution.	Typhoid Bacilli, Colonies on Plates in 5 Hours.		Serum in 24 Hours.		
		Normal Serum.	Typhoid Immune Serum.	Normal Serum.	Immune Serum.	
1.	1-1	0	Very many thousands.	Clear }	Growth } Pro-zone.	1-1
2.	1-2	1	" " "	Clear } Killing-zone.	Growth } Pro-zone.	1-2
3.	1-5	2	" " "	Clear } Killing-zone.	Growth } Pro-zone.	1-5
4.	1-20	2,500	4-5,000	Clear } Killing-zone.	Clear } Killing-zone.	1-20
5.	1-40	Many thousands.	4-5,000	Growth } Post-zone.	Clear } Killing-zone.	1-40
6.	1-80	Very many thousands.	Many thousands.	Growth } Post-zone.	Clear } Killing-zone.	1-80
7.	1-100	" " "	" "	Growth } Post-zone.	Growth } Post-zone.	1-100

Controls, colonies: at once, thousands.
Controls, colonies: in 5 hours, very many thousands.
The controls are made up like the other tubes, using salt solution instead of serum.

TABLE IX.

Paratyphoid.

	Serum 18 Hours Old Dilution.	Paratyphoid Bacilli, Colonies on Plates in 5 Hours.		Serum in 24 Hours.		
		Normal Serum.	Paratyphoid Immune Serum.	Normal Serum.	Immune Serum	
1.	1-1	0	Very many thousands.	Clear }	Growth } Pro-zone.	1-1
2.	1-2	0	" " "	Clear } Killing-zone.	Growth } Pro-zone.	1-2
3.	1-5	2,000	Many thousands.	Clear } Killing-zone.	Clear } Killing-zone.	1-5
4.	1-20	Many thousands.	" "	Clear } Killing-zone.	Clear } Killing-zone.	1-20
5.	1-40	Very many thousands.	" "	Growth } Post-zone.	Clear } Killing-zone.	1-40
6.	1-80	" " "	Very many thousands.	Growth } Post-zone.	Slight growth } Post-zone.	1-80
7.	1-100	" " "	" " "	Growth } Post-zone.	Slight growth } Post-zone.	1-100

Controls, colonies: at once, very many thousands.
Controls, colonies: in 5 hours, very many thousands.

The normal serum kills all the bacilli in each case up to a certain dilution (1–20), beyond which the bacilli are not affected. There is no pro-zone at all. The immune serum, on the contrary, has a definite killing-zone above and below which it is not bacteriolytic. The typhoid serum here kills typhoid bacilli at dilutions of 1–20 to 1–80, and paratyphoid serum kills paratyphoid bacilli from 1–5 to 1–40.

The result, therefore, is the same whether we dilute the immune bodies and keep the amount of complements fixed, as in the Neisser and Wechsberg experiments, or dilute both immune bodies and complements together. There is a pro-, killing-, and post-zone in each instance, so that the results predicted in Diagram III. on the basis of complement diversion are not realized, and in order to maintain the theory we are now forced to assume, in consequence of the results of experimental tests:

1. That complements are specific. Otherwise cholera bacilli would not be killed by typhoid immune serum, since general complements would be bound to the typhoid immune bodies.

2. That in concentrated typhoid immune serum complements may be diverted, but not in dilute immune serum, otherwise dilute immune serum would not kill typhoid bacilli any more than concentrated.

Before committing ourselves to such forced assumptions, we may consider if there is any other way of accounting for Neisser and Wechsberg's and analogous phenomena.

Shortly after Neisser and Wechsberg had published their views, Lipstein[1] investigated the subject and concluded that the phenomenon was certainly due to diversion of complements, and that the diversion of complements was not caused by:

(1.) Agglutination. Mechanical precipitation of complements.

[1] Centralblatt für Bakteriologie, Vol. 31, Orig., 1902, p. 460.

(2.) Normal anti-complements — as suggested by Metchnikoff.

(3.) Anti-complements formed in the immune serum — as suggested by Gruber.

Therefore complement diversion must be caused by combination of complements with the immune bodies.

His experiments and arguments need not be detailed since these points are of minor importance to us. They are merely mentioned because many German authors refer to Lipstein's experiments as having clinched the argument in favor of the theory.

Diversion of complements has not been observed in hemolytic serums, and Morgenroth explains this by supposing that in such cases the immune body when anchored to a red cell has more affinity for the complement than when it is free. Consequently the hemolytic immune bodies do not combine with complements except when previously anchored to a red cell.

However, Morgenroth[1] was able to obtain a phenomenon somewhat analogous to complement diversion by means of anti-immune bodies. On injecting serum of animal A, immunized to ox blood, into animal B, the latter developed anti-immune bodies, so that on treating ox corpuscles with immune serum A + anti-immune serum B + fresh complement-containing serum, the corpuscles were not dissolved.

The result was as depicted in Diagram IV., *a*, and the red cells remained unaffected because both immune bodies and complements were diverted from them by the anti-immune bodies.

Bordet[2] was able to confirm Morgenroth's observations, but maintains that he has drawn erroneous conclusions from his results. Bordet does not deny the possibility of anti-immune bodies being formed, but points out that on injecting any serum (in this case immune serum) into an animal it will produce anti-albumens directed against the albumens of the serum, and complements may be absorbed by these

[1] Centralblatt für Bakteriologie, Vol. 35, Orig., 1904, p. 501.

[2] Annales Pasteur, Vol. 18, 1904, p. 399.

anti-albumens just as well as by any supposititious anti-immune bodies.

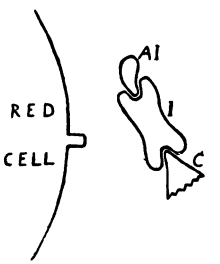
Wilde[1] and Gengou,[2] for instance, among others, have shown that complements can be absorbed by indifferent foreign albumens — dead bacteria, sterile pus, organ cells, etc., when these are added to serum. I find that cholera bacilli, killed at 60° C., will absorb the complements of normal rabbit serum for both typhoid and paratyphoid quite as readily as if the cholera bacilli were alive. In this case the cholera bacilli can simply represent inert foreign albumens.

A diagram explains the divergent views of Morgenroth and Bordet better than the description.

DIAGRAM IV.

Represents the conditions on mixing:

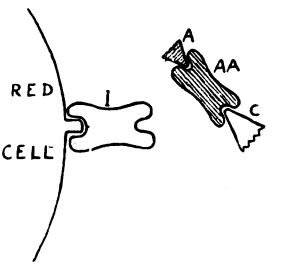
A. Serum immune to ox blood.
B. Serum immune to A, *i.e.*, anti-immune to ox blood.
C. Fresh normal serum to suppiy complements.
D. Ox corpuscles.



a.
According to Morgenroth.

AI. Anti-immune body of the anti-immune serum.
I. Immune body of the immune serum.
C. Complement of the normal serum.

The immune body is diverted from the red cell by the anti-immune body.



b.
According to Bordet.

A. Albumen of the immune serum.
AA. Anti-albumen of the anti-immune serum.
I. Immune body of the immune serum.
C. Complement of the normal serum.

The complement is diverted from the red cell by the anti-albumen.

Bordet has not applied his ideas to the phenomenon of complement diversion in bacterial immune serums, but in

[1] Berliner klinische Wochenschrift, 1901, p. 878.
[2] Annales Pasteur, Vol. 16, 1902, p 734.

view of his arguments for hemolytic serums it occurred to me that possibly some such process as he describes might account for the diversion of complements in typhoid immune serum.

There may be anti-albumens formed which occupy the complements to the exclusion of the immune bodies.

We know that typhoid bacilli possess certain easily detachable albumens, probably connected with the flagella, which may be split off by moderate heating (65° C. to 70° C.), shaking, etc. Filtrates from bacilli so treated will

1. Absorb agglutinin.
2. Produce agglutinating serums when injected into animals.
3. Form precipitates when treated with immune serum.

My own experiments on these lines have confirmed the observations of others.

The filtrates, therefore, must contain split-off products of typhoid bacilli, and Rossi [1] has recently maintained that he can demonstrate the presence of flagella in such filtrates by special methods of staining, although Nicolle [2] was unable to do so in spite of careful search.

On injection of typhoid bacilli into an animal we may suppose that these detachable albumens are broken off from the bacilli and cause the production of anti-bodies independently of the bacilli themselves.

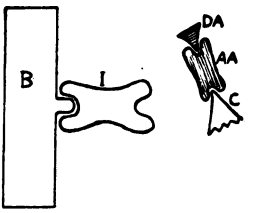
Such immune serums, therefore, would contain anti-albumens directed against the detachable albumens of the bacilli, as well as immune bodies directed against the bacilli themselves.

On adding typhoid bacilli to the immune serum we may suppose the detachable albumens to be broken off; the anti-albumens attach themselves to these on the one hand and to complements on the other, absorbing so many of the

[1] Centralblatt für Bakteriologie, Vol. 37, Orig., 1904, p. 438.
[2] Annales Pasteur, Vol. 18, 1904, p. 239.

complements that there are not sufficient left to deal with the bacilli (Diagram V.).

DIAGRAM V.



B. Bacillus.
DA. Detachable albumen.
AA. Anti-albumen.
I. Immune body.
C. Complement.

The complement occupies the anti-albumen, leaving the immune body incomplete.

Compare with the conditions obtaining according to the theory of complement diversion. Diagram I.

This, which we may call the anti-albumen theory, should be regarded as absorption rather than diversion of complements, and would account for Neisser and Wechsberg's phenomenon with a fixed amount of complement.

As the immune serum is diluted the anti-albumens become fewer in number, and a point is reached where they no longer absorb so many complements. The latter, therefore, are now able to occupy all the immune bodies, and, provided there are sufficient of these, the bacilli are destroyed (see Diagram VII., *a*, *b*, *c*).

This hypothesis would also account for the fact that typhoid immune serum will kill cholera bacilli. Since in this case no typhoid bacilli are added to the serum, there are no split-off products of the typhoid bacilli to which the anti-albumens can attach themselves. Consequently no complements are required to complete an albumen-anti-albumen combination, and they are all available for destruction of the cholera bacilli (see Diagram VI., *c*)

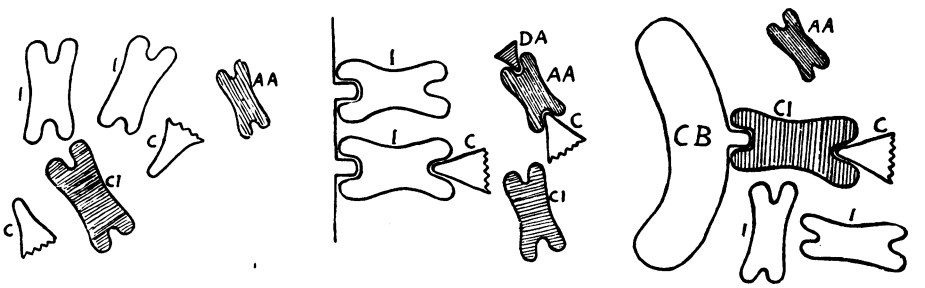
Accepting this theory of anti-albumens, we can also understand why concentrated fresh typhoid immune serum will not destroy typhoid bacilli, since in this case complements are

absorbed by the albumen-anti-albumen combination, the conditions being depicted in Diagram VI., *b*.

Diagram VI. explains the conditions obtaining in typhoid immune serum according to the anti-albumen theory.

It is assumed that it requires two I B–C combinations to kill the typhoid bacillus, but only I B–C combination to kill cholera. But there is no previous combination of complement and immune body, as has to be assumed for the theory of complement diversion. The complement does not attach itself to the immune body until after this has attached itself to the bacillus.

DIAGRAM VI.



DA. Albumen molecule detached from the typhoid bacillus.
AA. Anti-albumen.
I. Typhoid immune bodies.
CI. Cholera intermediate body.
C. Complement.

VI., *a*., shows the fresh typhoid immune serum before the addition of bacilli.

VI., *b*. A typhoid bacillus added to the serum.

An albumen molecule is split off from the typhoid bacillus and combines with the anti-albumen. The second complement required to kill the typhoid bacillus is absorbed by this combination, and the bacillus escapes destruction.

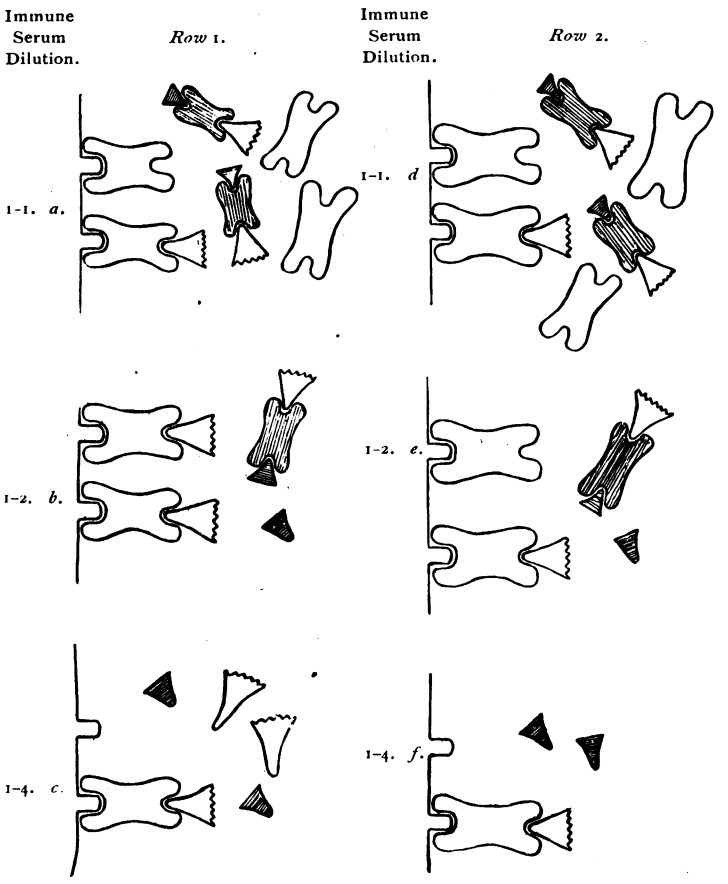
VI., *c*. A cholera bacillus added to the serum. The cholera intermediate body attaches itself to the bacillus, and one of the two available complements enters into the

combination. The cholera bacillus is killed. The anti-albumen, having no albumen molecule to combine with, is inactive.

But the anti-albumen theory will not account for the fact that *diluted* immune serum will kill its specific bacilli. On diluting the fresh immune serum the complements are diluted along with the anti-albumens and immune bodies, as already pointed out (see also Diagram VII., *d*, *e*, *f*), so that, as with the theory of complement diversion, we would have to assume a change in the affinities of the complements for the anti-bodies consequent on dilution of the serum.

In this case the affinities of the complements for the anti-albumens would have to be so much weakened by dilution that they are now able to devote all their energies to the immune bodies and consequently can destroy the bacilli. This is an assumption we would hardly be justified in making.

DIAGRAM VII. — *Anti-albumen Theory.*



The detachable albumens and the anti-albumens are figured with stripes.

Row 1 represents the conditions in the Neisser and Wechsberg experiments with a fixed amount of the complement; in this case we suppose three complements at each dilution of the immune serum.

It is obvious that there is here a pro-(1–1), killing-(1–2), and post-zone (1–4), as with the theory of complement diversion.

Row 2. The conditions on diluting fresh immune serum,

There should be no killing-zone in this instance, as a glance at the diagram will show. There is no opportunity for two complements to enter into relation with the bacillus. Since, however, we already know that there is a killing-zone, it seemed unlikely from theoretical considerations that the anti-albumen theory would furnish a clue to the phenomena, but nevertheless some experiments were made in an endeavor to test it.

EXPERIMENTS. Anti-albumens. — Typhoid bacilli were suspended in distilled water, shaken in a shaking machine for one hour, the water filtered off through a Berkefeld filter, the bacilli thoroughly washed and suspended in salt solution. If the detachable albumens have been removed, the bacilli should now be more susceptible to concentrated fresh immune serum than normal bacilli, since no detachable albumens will be liberated to absorb complements. Several experiments were made to test this, and the accompanying tables serve as illustrations of the results obtained.

TABLE X.

	Normal Typhoid Bacilli.				Shaken and Washed Typhoid Bacilli.			
	Normal Serum Dilution.	Typhoid, Colonies in 5 Hours.	Serum in 24 Hours.		Serum in 24 Hours.	Typhoid, Colonies in 5 Hours.	Normal Serum Dilution.	
1.	1-2	Few.	Clear		Killing-zone: Clear	Hundreds.	1-2	.1
2.	1-5	Hundreds.	Clear	Killing-zone.	Killing-zone: Clear	"	1-5	.2
3.	1-20	Many thousands.	Clear		Killing-zone: Clear	Many thousands.	1-20	.3
4.	1-40	Very many thousands.	Growth	Post-zone.	Post-zone: Growth	Very many thousands.	1-40	.4
5.	1-80	" " "	Growth		Post-zone: Growth	" " "	1-80	.5

	Normal Typhoid Bacilli.				Shaken and Washed Typhoid Bacilli.			
	Serum, Typhoid Immune.	Typhoid, Colonies in 5 Hours.	Serum in 24 Hours.		Serum in 24 Hours.	Typhoid, Colonies in 5 Hours.	Serum Typhoid Immune.	
1.	1-1	Many thousands.	Growth	Pro-zone.	Pro-zone: Growth	Many thousands.	1-1	.1
2.	1-5	" "	Clear	Killing-zone.	Killing-zone: Clear	" "	1-5	.2
3.	1-25	Very many thousands.	Growth		Post-zone: Growth	" "	1-25	.3
4.	1-50	" " "	Growth	Post-zone.	Post-zone: Growth	Very many thousands.	1-50	.4
5.	1-100	" " "	Growth		Post-zone: Growth	" " "	1-100	.5

Controls, normal bacilli: at once, many thousands; in 5 hours, very many thousands.
Controls, shaken bacilli: at once, many thousands; in 5 hours, very many thousands.
In order that the most important columns, those showing the condition of the serum in 24 hours, may be readily compared, the second series has been given in reversed order.

It is obvious that the shaken and washed typhoid bacilli are not more susceptible than normal bacilli either to the normal or immune typhoid serum. The normal serum kills in each case up to one to twenty dilution, and the killing-zone for the immune serum is the same for each.

Table XI. shows the same results with paratyphoid, although in this particular instance the bacilli were sown too thickly, so that no actual killing-zone appears with the

immune serum. Still, at dilutions of one to twenty-five and one to fifty, the colonies on the plates in five hours and the growth in the serum in twenty-four hours were distinctly less than at lower and higher dilutions, so that the table serves very well for purposes of comparison.

TABLE XI.

	Normal Paratyphoid Bacilli.			Shaken and Washed Paratyphoid Bacilli.			
	Serum, Normal	Paratyphoid, Colonies in 5 Hours.	Serum in 24 Hours.	Serum in 24 Hours.	Paratyphoid, Colonies in 5 Hours.	Serum, Normal.	
1.	1-2	Few.	Clear.	Clear.	Few.	1-2	.1
2.	1-5	Many thousands.	Growth.	Growth.	Thousands.	1-5	.2
3.	1-20	" "	"	"	Very many thousands.	1-20	.3
4.	1-80	Very many thousands.	"	"	" " "	1-40	.4
5.	1-100	" " "	"	"	" " "	1-80	.5

	Normal Paratyphoid Bacilli.			Shaken and Washed Paratyphoid Bacilli.			
	Serum, Paraty-phoid Immune	Paratyphoid, Colonies in 5 Hours.	Serum in 24 Hours.	Serum in 24 Hours.	Paratyphoid, Colonies in 5 Hours.	Serum, Paraty-phoid Immune	
1.	1-1	Very many thousands.	Growth.	Growth.	Very many thousands.	1-1	.1
2.	1-5	" " "	"	"	" " "	1-5	.2
3.	1-25	Many thousands.	Slight Growth.	Slight Growth.	Many thousands.	1-25	.3
4.	1-50	" "	" "	" "	" "	1-50	.4
5.	1-100	Very many thousands.	Growth.	Growth.	Very many thousands.	1-100	.5

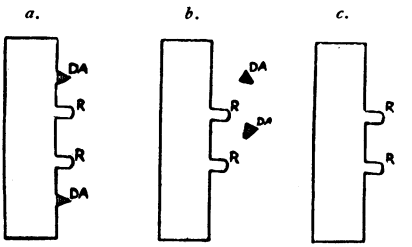
Controls, normal bacilli: at once, very many thousands.
Controls, normal bacilli: in 5 hours, very many thousands.
Controls, shaken bacilli: at once, very many thousands.
Controls, shaken bacilli: in 5 hours, very many thousands.

We find, then, that the shaken and washed bacilli do not appear to be more susceptible to concentrated immune serum than normal bacilli, but possibly shaking and washing does not sufficiently strip the bacilli of their detachable albumens. It may be that the immune serum itself has the power of doing this more effectively. If, therefore, bacilli are treated

with immune serum they should, provided the anti-albumen theory is sound, become completely stripped of their detachable albumens, and after washing free of the serum should be readily killed by fresh concentrated immune serum (Diagram VIII.). If they are not killed we must assume that immune serum has no power of stripping the bacilli, and the anti-albumen theory falls to the ground. One cannot suppose that without this supposititious stripping of the bacilli antibodies, capable of absorbing complements, can be formed other than those directed against the bacilli themselves.

DIAGRAM VIII.

Representing the condition of normal and stripped bacilli.



R. Receptors.
DA. Detachable albumens.

VIII., *a*. Normal (typhoid) bacillus with two detachable albumen molecules (DA) and two receptors (R).

VIII., *b*. Bacillus on introduction into immune serum. The two albumen molecules are detached by the action of the serum.

VIII., *c*. Bacillus represented in *b* after washing free of the immune serum. The detached albumens have been got rid of. The bacillus in this condition should be susceptible to the action of concentrated immune serum, since there are no detachable albumens left to absorb complement.

EXPERIMENTS. — To test the stripping power of immune serum.

To five cubic centimeters of fresh immune serum, one

cubic centimeter of an emulsion of typhoid (resp. paratyphoid) bacilli was added, and the mixture allowed to stand for two hours in the incubator.

The bacilli, as we have seen, will not be killed. The question is, Will they be stripped?

At the end of the two hours the mixture was centrifugalized, the supernatant clear serum decanted off, the sedimented bacilli washed three times with salt solution, and a thin emulsion of them made in salt solution.

For controls an emulsion of normal bacilli was made, care being taken to get the two emulsions of, as nearly as possible, the same opacity.

The tubes were made up and inoculated as already described, two cubic centimeters of fluid in each tube, to which five drops of the emulsion were added.

Table XII. is selected as typical of the experiments with typhoid and Table XIII. of paratyphoid.

TABLE XII.

Typhoid serum and typhoid bacilli.

	Normal Typhoid Bacilli.				Treated and Washed Typhoid Bacilli.			
	Serum, Typhoid Immune Dilution.	Typhoid, Colonies in 5 Hours.	Serum in 24 Hours.		Serum in 24 Hours.	Typhoid, Colonies in 5 Hours.	Serum, Typhoid Immune Dilution.	
1.	1–1	Many thousands.	Growth	Pro-zone.	Pro-zone: Growth	Very many thousands.	1–1	·1
2.	1–2	" "	Growth		Pro-zone: Growth	" " "	1–2	·2
3.	1–5	" "	Growth		Pro-zone: Growth	" " "	1–5	·3
4.	1–10	" "	Growth		Pro-zone: Growth	" " "	1–10	·4
5.	1–20	Thousands.	Clear	Killing-zone.	Killing-zone: Slight growth	Many thousands.	1–20	·5
6.	1–40	Many thousands.	Very slight growth	Killing-zone.	Killing-zone: Slight growth	Very many thousands.	1–40	·6
7.	1–80	" "	Growth	Post zone.	Post-zone: Growth	" " "	1–80	·7

Controls, normal bacilli: at once, 6,000.
Controls, normal bacilli: in 5 hours, very many thousands.
Controls, treated bacilli: at once, 3,000.
Controls, treated bacilli: in 5 hours, very many thousands.

The bacilli, previously treated with immune serum and washed, appear to be somewhat more resistant to the action of fresh immune serum than the untreated bacilli. The latter are all killed in twenty-four hours at 1–20, whereas the treated bacilli have increased slightly at this dilution. At lower dilutions both show the same pro-zone.

TABLE XIII.

Paratyphoid serum and paratyphoid bacilli.

	Normal Paratyphoid Bacilli.			Treated and Washed Paratyphoid Bacilli.			
	Serum, Paratyphoid Immune Dilution.	Paratyphoid Colonies in 5 Hours.	Serum in 24 Hours.	Serum in 24 Hours.	Paratyphoid, Colonies in 5 Hours.	Serum, Paratyphoid Immune Dilution.	
1.	1–1	Very many thousands.	Growth (Pro-zone.)	Pro-zone: Growth	Very many thousands.	1–1	.1
2.	1–2	Many thousands.	Growth (Pro-zone.)	Pro-zone: Growth	Thousands.	1–2	.2
3.	1–5	Hundreds.	Clear (Killing-zone.)	Pro-zone: Growth	Hundreds.	1–5	.3
4.	1–10	Few hundreds.	Clear (Killing-zone.)	Killing-zone: Clear	Few hundreds, 200.	1–10	.4
5.	1–20	Hundreds.	Clear (Killing-zone.)	Killing-zone: Clear	Hundreds.	1–20	.5
6.	1–40	Thousands.	Growth (Post-zone.)	Post-zone: Growth	Thousands.	1–40	.6
7.	1–80	Very many thousands.	Growth (Post-zone.)	Post-zone: Growth	Thousands.	1–100	.7

Controls, normal bacilli: at once, many thousands.
Controls, normal bacilli: in 5 hours, very many thousands.
Controls, treated bacilli: at once, thousands.
Controls, treated bacilli: in 5 hours, very many thousands.

Here also the treated bacilli are certainly not more susceptible than the untreated. On the contrary, they are slightly more resistant to the action of concentrated serum, as shown by growth in the serum in twenty-four hours at one to five dilution, whereas at this dilution the normal bacilli were all killed.

There is no evidence either with typhoid or paratyphoid to show that the treated bacilli have been stripped of detachable albumens, which, through the medium of anti-albumens, could absorb complements.

We must therefore conclude from all the data thus far brought forward that:

A. The theory of diversion of complements will account for:

1. Löffler and Abel's observations that an excess of immune serum will not protect a normal animal (Table I.).

2. Neisser and Wechsberg's phenomenon "in vitro" with a fixed amount of complement (Tables II. and III.).

3. The non-bacteriolytic power of concentrated immune serum (Tables IV. and V.).

But it will not account for:

4. Concentrated typhoid (or paratyphoid) immune serum being bacteriolytic for cholera bacilli (Table VII.).

5. The killing-zone on dilution of fresh immune serum, above and below which the immune serum has no bacteriolytic action (Tables VII. and IX.).

B. The theory of absorption of complements by anti-albumens does not stand experimental tests (Tables X. to XIII.), though theoretically it might account for:

1. Löffler and Abel's observations.

2. Neisser and Wechsberg's phenomenon.

3. Non-bacteriolytic power of concentrated immune serum.

4. That concentrated typhoid immune serum is bacteriolytic for cholera bacilli.

But it would not account for:

5. The killing-zone on dilution of fresh immune serum.

Yet it seems probable that all the phenomena discussed must have a common cause, so that unless we are prepared to make the very forced assumptions previously alluded to we must reject both these hypotheses as being untenable.

Is there any other hypothesis which will account for these phenomena in a more satisfactory manner? The last two tables, XII. and XIII., seem to indicate that bacilli, previously treated with immune serum, and therefore strongly agglutinated, are less susceptible to the influence of immune serum

than normal bacilli, and it seems possible that the apparently non-bacteriolytic power of concentrated immune serum may be due to the fact that the bacilli are so quickly and strongly agglutinated that they are protected to some extent from the action of the lysins. This point of view will be fully discussed in the second part of this article.