ON SOME FACTORS IN BACTERIOLYTIC ACTION[1].

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That blood or serum and other body fluids, such as the natural or artificially excited exudations, possess bacteriolytic power is a familiar observation.

As the result of a series of experiments carried out on guinea-pigs in Berne early last year (1901) I was able to publish[2] evidence confirmatory of the fact originally observed by Nuttall[3] nearly 15 years ago, that the supposed ferment (complement, addiment) upon which this power depends suffers a rapid disappearance from the fluids in question after their removal from the living body. The experiments also showed that a complement available for guinea-pigs and at the same time satisfactory to the immune body which was used for their protection—that of horses immunised against the *Bacillus typhosus*—was supplied by the fresh serum of all the species of animals examined (ox, sheep and pig), and therefore is not so special to the species as was originally held by Professor Ehrlich. And this complement I found to exist not only in the serum, but also in fresh clot from which the serum had been separated; a fact which lends support to the contention that the bacteriolytic ferment is of leucocytic origin.

These observations have now been followed up in an examination of the bacteriolytic power of blood and serum in the test-tube; more

[1] A preliminary note of this paper was published in *The Lancet* of Jan. 4th, 1902, p. 18, entitled "The Disappearance of the Addiment from Anti-Microbic Sera." Its publication in full has been unavoidably delayed. (MS. received 4. xii. 1902. *Ed.*)

[2] *Journal of Hygiene*, 1902, Vol. ii. p. 85, "On the Protective Substances of Immune Sera."

[3] *Zeitschr. f. Hygiene*, 1888, Bd. iv. pp. 353—394.

especially with reference to the time relations which are found to exist, and the quantitative influence exerted by the immune body upon the process of bacteriolysis.

Methods.

The plan of the experiments was arranged as follows. A number of rabbits were immunised by the successive injection of increasing doses of living typhoid culture, and were subsequently bled for the preparation of their sera. The weights and temperatures of the animals were recorded daily. Three of the animals instead of rapidly regaining the temporary loss of weight which followed each injection of typhoid bacilli became distinctly ill with loss of appetite, languor and wasting. They were intentionally bled and killed at an unfavourable period in the immunity reaction in order to determine to what extent the bacteriolytic action of their sera was affected by their condition.

The immunisation of the rabbits is shown in Table I. below. The animal experiments were done in Berne last year (1901) in the laboratory of Professor Tavel, to whom I was again indebted for the opportunity of working there.

The sera yielded by the rabbits were examined for bacteriolytic action on broth cultures of the *Bacillus typhosus* of a definite age, and were compared in this respect with the fresh sera of five normal rabbits. The following procedure was employed. The blood was taken by means of a sharp-pointed metal cannula from the exposed femoral artery[1], and was received into sterile test-tubes and allowed to clot, the clot being subsequently loosened a little from the side of the tube by means of a stout platinum needle in order to assist the separation of the serum.

So soon as fluid began to separate from the clot a specimen was removed for examination, and after an interval of from four to six hours from the time of bleeding a second specimen was taken. At the end of 24 hours the remainder of the serum was removed and placed in sterile tubes for later use.

Anti-bacterial action was tested as follows: the serum to be examined was measured out by means of a sterilised pipette from which a given number of drops were allowed to fall into a sterile test-tube. The same pipette was always used throughout each series of

[1] The insertion of the cannula requires a little dexterity, but the method has the advantage of ensuring sterility and yielding a relatively large amount of blood.

TABLE I.

Immunisation of Rabbits.

Rabbit	Day 1	Day 3	Day 5	Day 10	Day 14	Day 19	Day 2
I. *a.*	$\frac{1}{20}$th of a 24-hours old agar culture subcutaneously	$\frac{1}{20}$th of a 24-hours old agar culture subcutaneously	$\frac{1}{10}$th of a 48-hours old agar culture subcutaneously	Bled	——	——	——
I. *b.*	,, ,,	,, ,,	,, ,,	$\frac{1}{10}$th of a 48-hours old agar culture subcutaneously	Bled	——	——
I. *c.*	,, ,,	,, ,,	,, ,,	,, ,,	,,	——	——
I. *d.*	,, ,,	,, ,,	,, ,,	——	——	——	Bled
I. *e.*	,, ,,	,, ,,	,, ,,	$\frac{1}{10}$th of a 48-hours old agar culture subcutaneously	——	——	,,
I. *f.*	,, ,,	,, ,,	,, ,,	,, ,,	$\frac{1}{5}$th of a 48-hours old agar culture subcutaneously	Bled	——
I. *g.*	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,,	——
I. *h.*	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	——	Bled
I. *k.*	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	——	,,
I. *m.*	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	——	,,

TABLE II. *Showing the number of Colonies in the Typhoid Plates from Rabbits' sera.*

Age of serum	Drops of serum	Typhoid culture	N.1.	N. 2.	N. 3.	N. 4.	N. 5.	I. *a.*	I. *b.*	I. *c.*	I. *d.*	I. *e.*	I. *f.*	I. *g.*	I. *h.*	I. *k.*	I. *m.*
1—2	5	1 loopful	—	—	—	—	—	—	—	—	450	2	—	—	3,500	5,000	16,000
hours	10	,,	1,000	800	—	—	—	300	600	300	300	1	—	—	1,600	2,500	8,000
	20	,,	400	250	—	—	—	100	250	100	—	—	—	—	—	—	—
	30	,,	250	70	—	—	—	—	150	20	—	—	—	—	—	—	—
4—6	5	,,	—	—	1,500	3,500	2,000	—	—	—	3	3	—	—	3,000	3,500	4,000
hours	10	,,	3,000	600	700	1,500	800	20	600	100	1	1	—	—	1,400	1,800	1,200
	20	,,	2,000	120	—	—	—	10	300	35	—	—	—	—	—	—	—
	30	,,	1,000	40	—	—	—	—	150	10	—	—	—	—	—	—	—
24 hrs.	5	,,	—	—	8,000	9,000	10,000	—	—	—	70	800	—	—	30,000	25,000	40,000
	10	,,	5,000	1,000	3,000	4,000	4,500	300	2,000	1,500	20	400	—	—	10,000	6,000	6,000
	20	,,	2,500	200	—	—	—	100	800	500	—	—	—	—	—	—	—
	30	,,	—	50	—	—	—	—	300	200	—	—	—	—	—	—	—
2 days	5	,,	—	—	20,000	unc.	unc.	—	—	—	2,000	7,000	600	30,000	unc.	50,000	unc.
	10	,,	16,000	5,000	7,000	20,000	30,000	20,000	30,000	15,000	800	3,000	30	25,000	unc.	30,000	35,000
	20	,,	9,000	1,500	—	—	—	10,000	12,000	7,000	—	—	—	—	—	—	—
	30	,,	—	300	—	—	—	—	—	—	—	—	—	—	—	—	—
3 ,,	5	,,	—	—	50,000	∞	∞	—	—	—	10,000	55,000	2,500	unc.	∞	unc.	∞
	10	,,	unc.	8,000	30,000	unc.	unc.	unc.	unc.	50,000	2,500	40,000	100	unc.	∞	unc.	unc.
	20	,,	unc.	5,000	—	—	—	unc.	unc.	30,000	—	—	—	—	—	—	—
4 ,,	5	,,	—	—	unc.	∞	∞	—	—	—	50,000	unc.	12,000	∞	∞	∞	∞
	10	,,	∞	40,000	unc.	∞	∞	∞	∞	unc.	30,000	unc.	8,000	∞	∞	∞	∞
	20	,,	∞	—	—	—	—	∞	∞	unc.	—	—	—	—	—	—	—
5 ,,	5	,,	—	—	∞	—	—	—	—	—	unc.	∞	30,000	∞	—	∞	∞
	10	,,	—	unc.	∞	—	—	∞	∞	∞	unc.	∞	20,000	∞	—	∞	∞
	20	,,	—	—	—	—	—	∞	∞	∞	—	—	—	—	—	—	—
6 ,,	5	,,	—	—	∞	—	—	—	—	—	∞	—	unc.	∞	—	—	—
	10	,,	—	∞	∞	—	—	—	—	∞	∞	—	unc.	∞	—	—	—
	20	,,	—	—	—	—	—	—	—	∞	—	—	—	—	—	—	—
8 ,,	5	,,	—	∞	—	—	—	—	—	—	∞	—	∞	—	—	—	—
	10	,,	—	∞	—	—	—	—	—	∞	∞	—	∞	—	—	—	—
	20	,,	—	—	—	—	—	—	—	∞	—	—	—	—	—	—	—

unc. = uncountable: ∞ = infinite

observations, so that the succeeding observations in each series are strictly comparable; and all the observations given in Table II. were carried out by means of the same pipette and are therefore comparable throughout. These pipettes were blown for the purpose and were drawn out in such a way as only to allow the escape of fluid in drops even when the finger was entirely removed from the upper end. The size of the drops from any one pipette was therefore approximately constant. In order to obtain an estimate of the amounts of serum that were being used, a number of the pipettes were measured as regarded the volume of the drops which they allowed to escape. And it was found that the volume of 300 drops varied with different pipettes between 8·6 and 9·4 cubic centimetres, the average being about 9 cubic centimetres. Twenty drops were therefore equivalent to about 0·6 cubic centimetres of the fluid employed.

The tubes of serum which had been measured in the manner just described were next inoculated each with one loopful of a culture of typhoid bacilli grown for about 24 hours in 10 cubic centimetres of bouillon. The tubes were incubated at 37° C. for a period of three hours[1], to each was then added 10 cubic centimetres of melted gelatin culture-medium, and the contents thoroughly mixed and plated. Colonies were counted in the plates after three days' growth. In every case a control plate was made with uninoculated serum to test its sterility, and another with one loopful of the typhoid culture inoculated and incubated for three hours in 0·5 cubic centimetres of ordinary bouillon for comparison with the plates from inoculated serum.

The colonies were enumerated by means of a Wolffhügel counting apparatus. Where they were very numerous they were estimated approximately. Up to 100 or more colonies per square cm. could be counted with a lens with moderate accuracy. Above this number I have used the term "uncountable," where the colonies were obviously less numerous than those in the control without serum, and the sign ∞ (infinite) where no such difference was appreciable.

The results of the first series of observations made on fresh rabbits' serum are given in Table II.

In the foregoing Table II., N. 1 to N. 5 are the sera of the five normal rabbits, and I. *a.* to I. *m.* are the sera of the rabbits which had been inoculated with the *Bacillus typhosus* as shown in Table I.

[1] The time three hours was chosen for convenience because it was found that at this period the number of colonies which grew in the control plates from ordinary bouillon had become innumerable, and therefore formed a constant standard for comparison.

1. The Table II. shows quantitatively the progressive disappearance of the complement (addiment) from separated serum. The fact that complement thus suffers a rapid disappearance is of course an old observation, but I am unable to discover that any attempt has previously been made to study the time relations of the process.

2. The Table also shows that while there is some difference between different sera in the rapidity with which the complement disappears, no evident differences appear between the sera of normal and of immunised animals in this respect.

3. The bacteriolytic power *in vitro* of the sera of the immunised animals was markedly greater than that of the normal sera, except in the case of I. *h.*, I. *k.*, and I. *m.* These were the sera of the animals already referred to which were killed while in a condition of pyrexia and impaired nutrition following the inoculations. In them the bacteriolytic action of the serum was very definitely less than that of normal uninoculated animals.

4. I. *f.* and I. *g.* were the sera of two rabbits of about the same age and weight, which had been similarly treated (see Table I.) and which were bled at the same time. The serum I. *f.* was kept continuously in an ice-chest; I. *g.* and all the other sera being kept at the ordinary room-temperature of the laboratory (about 14° C.) but in the dark. The serum I. *f.* was not separated from the clot at the end of 24 hours as were the other sera but was left in contact with its clot throughout, only so much as was required for observation being removed each day. It shows a marked retardation of the disappearance of the bacteriolytic power.

On comparing the bacteriolytic power of serum taken from the clot from one to two hours after the blood had been collected with that of serum of the same animal removed from two to four hours later (*i.e.* four to six hours old) from the same tube a very remarkable fact was to be observed in many of the sera, namely, that *the bacteriolytic power was greater at the second observation than at the first.* That is to say that the amount of complement in the serum had apparently undergone an increase during the early hours of the separation of the serum from the clot. In order to verify this observation I have proceeded to examine a number of normal sera such as could be readily obtained in quantity, using the same method as before and estimating their bacteriolytic action at each succeeding hour. Sheep's serum was the most easily available, and several specimens of this were obtained on different occasions and tested hourly for the first six hours from the

time of slaughtering. This with the subsequent three hours' incubation followed by plating requires from nine to ten hours continuous work, and therefore as the sheep were only killed at the slaughter-house between 3 and 4 o'clock in the afternoon it was not found convenient to extend the observations further the same night.

As already stated the method followed was the same as in the case of the rabbits' sera above, but here the typhoid culture was used in each

TABLE III.

Serum and whipped blood of sheep, showing the number of colonies which grew in each plate.

		Streptococcus Plates		Bacillus Typhosus Plates				
Age of serum or whipped blood	Quantity of serum or whipped blood	Strepto-coccus culture about 8 hours old	Colonies from serum tubes of sheep 1	Typhoid culture about 8 hours old	Colonies from serum tubes of sheep 1	Colonies from serum tubes of sheep 2	Colonies from whipped blood tubes of sheep 3	Colonies from whipped blood tubes of sheep 4
1 hour	20 drops	1 loopful	100,000	1 loopful	65	35	8	10
2 hours	,,	,,	44,100	,,	23	20	11	10
3 ,,	,,	,,	37,800	,,	19	12	9	13
4 ,,	,,	,,	33,500	,,	16	23	15	17
5 ,,	,,	,,	31,900	,,	13	28	22	21
6 ,,	,,	,,	36,700	,,	17	33	31	33
18 ,,	,,	—	—	,,	—	62	—	—
20 ,,	,,	—	—	,,	51	—	—	—
4 hours taken from clot at 1 hour	,,	—	—	,,	—	34	—	—
5 hours taken from clot at 1 hour	,,	—	—	,,	—	42	—	—
5 hours taken from clot at 2 hours	,,	—	—	,,	35	—	—	—
5 hours taken from clot at 3 hours	,,	—	—	,,	26	—	—	—
18 hours taken from clot at 5 hours	,,	—	—	,,	—	495	—	—

Control plates of uninoculated serum all sterile.

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case, one planted in broth early on the morning of the experiment and incubated until required the same evening. On some occasions the sera were tested also against cultures of *Streptococcus*. The serum was left in contact with the clot throughout, a sample being removed each hour for examination. In some cases also some of the serum was removed from the clot after one hour or several hours and left to stand in a test-tube for comparison at a later period with a sample of serum freshly removed from the flask containing clot and serum. On two occasions instead of serum whipped blood was obtained and submitted to a similar examination. The most satisfactory method of obtaining sterile whipped blood for this purpose was found to be by receiving the blood into an Erlenmeyer flask into which a small spiral coil of iron wire had been introduced before sterilisation, the flask being kept in gentle agitation until the deposition of fibrin was completed.

In Tables III. and IV. the results obtained with two sheep sera and with whipped blood from two other sheep are recorded, as well as those with the serum and whipped blood of the same rabbit.

TABLE IV.

Serum and whipped blood of Rabbit A, showing the number of colonies which grew in each plate.

Age of serum or whipped blood	Quantity of serum or whipped blood	Typhoid culture about 16 hours old	Colonies from serum tubes	Colonies from whipped blood tubes
1 hour	20 drops	1 loopful	600	150
2 hours	,,	,,	480	170
3 ,,	,,	,,	350	210
4 ,,	,,	,,	240	280
5 ,,	,,	,,	270	340
6 ,,	,,	,,	330	430
6 hours taken from clot at 1 hour	,,	,,	810	—
6 hours	,,	0	0	0

These Tables (III. and IV.) show incidentally the very marked difference between the bacteriolytic power of normal sheep's blood for typhoid bacilli and that for *Streptococci*. They also show that while the bacteriolytic power of whipped blood is originally somewhat greater than the maximum attained by serum (cp. Table IV.) yet it diminishes progressively with each succeeding hour, while on the other

hand *the bacteriolytic power of serum which is left in contact with the clot progressively increases during the earlier hours after the blood is shed, and only subsequently begins to undergo a diminution.* And that this increase is associated with the presence of the clot follows from a comparison of the observations on specimens of serum taken from the flask containing the clot at a given hour but only tested at a later period after some hours' standing with those on specimens freshly removed from the clot-containing vessel and submitted to examination at the same later period as the former. Thus to take the two specimens of serum in Table IV. which were examined for bacteriolytic power at the sixth hour, the first, which was freshly taken from the clot for this examination, only showed 330 colonies on plating after the usual procedure, while the second, which had been removed at the first hour—at a time when the bacteriolytic power was such that some 600 colonies were formed upon the plate from serum examined at that age—and had been left to stand in a test-tube for the next five hours, allowed the growth of 810 colonies after similar treatment.

Nuttall[1] in 1888 was the first to work upon the bacteriolytic action of fresh serum. He showed that lysogenesis depends upon some property of the serum which is destroyed by the application of a temperature of from 52° to 55° C. Following the appearance of Nuttall's paper came a numerous series of experiments by Buchner[2], Lubarsch and others, in none of which, however, does any exact consideration of the age of the serum used appear to have been taken into account. Behring and Nissen[3] have also published numerous results of their experiments on the bacteriolytic action of fresh sera. These sera were usually employed of the age of two, four, or six hours from the time of bleeding, but no comparison was made of the action of one and the same serum at succeeding periods. Von Fodor[4] showed that in defibrinated dog's blood the bacteriolytic power for anthrax steadily decreases from the time the blood is shed. He concluded that this progressive loss is due to changes leading to the production of acid in the shed blood; and showed that the bacteriolytic power of blood may be increased by previous administration of various alkaline substances, which he believed delayed this gradual process of acidification. The salts which von Fodor

[1] *Zeitschrift für Hygiene*, 1888, IV. p. 353.

[2] *Centralblatt für Bakteriologie*, 1899, V. and VI. *Fortschritte der Medicin*, 1892, Vol. X. pp. 9, 10.

[3] *Zeitschrift für Hygiene*, 1890, VIII.

[4] *Centralblatt für Bakteriologie*, 1890, VII. p. 753.

administered were however such as have also the effect of producing a considerable leucocytosis.

Considering the results of the experiments which I have here recorded, it seems quite clear that while in a whipped blood the whole available bacteriolysin of the blood is of course present from the outset, and undergoes a steady diminution or degeneration from the first; that of the serum while progressively deteriorating also, is in the earlier hours continually receiving fresh additions from the clot. Accordingly the evidence supports most strikingly the view that *the bacteriolytic "ferment" is a leucocytic product*, and is yielded to the serum by the gradual disintegration of the leucocytes during and subsequently to coagulation of the blood. Moreover this accords entirely with the experiments which have been published by Bordet and Gengou[1] showing that *plasma* rapidly separated without appreciable destruction of leucocytes has practically no bacteriolytic action. It is completely in antagonism with the view which was proposed by Ehrlich that the bacteriolytic "ferment" is a substance normally present in the plasma of the living animal.

During the progress of the first series of observations (*vide* Table I.) I was anxious to determine how far, if at all, the amount of immune body produced by the inoculated rabbits exceeded their available complement (addiment). Accordingly I took a series of tubes containing equal amounts of fresh rabbits' serum of six hours' age and added to them different quantities of rabbits' immune serum which had been heated for an hour at 55° C. The tubes were then inoculated, incubated and plated in the usual way. Some of the plates gave the unlooked-for result which is shown in Table V. below.

TABLE V.

Showing the effect on bacteriolytic power of the addition to fresh serum of increasing quantities of inactivated immune serum.

Fresh rabbits' serum	Serum I. *q.* heated for 1 hour at 55° C.	Typhoid culture	Colonies in plates
10 drops	2 drops	1 loopful	360
,,	5 ,,	,,	1,920
,,	10 ,,	,,	48,000
,,	0	0	0

[1] *Annales de l'Institut Pasteur*, 1901.

Here it appeared that the more immune serum had been added to the fresh normal serum the greater was the number of colonies which grew in the plates; in other words, that an excess of immune body had the effect of lessening the bacteriolytic action exercised.

This fact I found had been already recorded by Neisser and Wechsberg[1], who (1901) state that both in the living animal and in the test-tube an excess of immune serum is prejudicial to bacteriolytic action. I have since made a number of observations on this question

TABLE VI.

Showing the effect upon bacteriolysis of the addition to fresh serum of increasing quantities of inactive immune serum.

Fresh rabbits' serum	Inactive rabbits' antityphoid serum. Drops	Typhoid culture	Colonies in plates
10 drops	0	0	0
,,	2	1 loopful	400
,,	5	,,	1,800
,,	10	,,	45,000
,,	20	,,	unc.
,,	40	,,	∞
,,	80	,,	∞
,,	0	,,	830
5 drops	0	,,	1,960

unc. = uncountable : ∞ = infinite.

TABLE VII.

Showing the effect upon bacteriolysis of the addition to fresh serum of a rabbit of increasing quantities of inactive immune serum of a horse.

Fresh rabbits' serum	Inactive horse's antityphoid serum. Drops	Typhoid culture	Colonies in plates
10 drops	0	0	0
,,	0	1 loopful	1,080
,,	1	,,	420
,,	5	,,	1,320
,,	25	,,	50,000
,,	50	,,	unc.
,,	100	,,	∞

unc. = uncountable : ∞ = infinite.

[1] *Münchener medicinische Wochenschrift*, 1901, Vol. XLVIII. p. 679.

with the fresh sera of the rabbit and the sheep and old[1] immune sera of the rabbit and the horse. The results, which serve to confirm the discovery of Neisser and Wechsberg, are given in Tables VI. VII. and VIII.

TABLE VIII.

Showing the effect upon bacteriolysis of the addition to the fresh sera of sheep 1 *and* 2 *of increasing quantities of inactive immune serum of the rabbit and of the horse.*

	Fresh serum 4 hours old. Drops	Inactive rabbit's antityphoid serum. Drops	Typhoid culture	Colonies in plates	Fresh serum 4 hours old. Drops	Inactive horse's antityphoid serum. Drops	Typhoid culture	Colonies in plates
Fresh serum of sheep 1	20	0	0	0	—	—	—	—
	0	20	0	0	0	20	0	0
	20	0	1 loopful	15	20	0	1 loopful	17
	0	20	,,	∞	0	20	,,	∞
	20	1	,,	12	20	1	,,	0
	20	5	,,	2	20	5	,,	10
	20	10	,,	20	20	10	,,	300
	20	20	,,	420	20	20	,,	3,600
	20	40	,,	5,400	20	40	,,	27,000
	20	60	,,	32,400	20	60	,,	70,000
	20	80	,,	54,000	20	80	,,	unc.
	20	100	,,	unc.	20	100	,,	∞
	20	140	,,	∞	20	150	,,	∞
Fresh serum of sheep 2	20	0	0	0	—	—	—	—
	0	20	0	0	—	—	—	—
	20	0	1 loopful	30	20	0	1 loopful	28
	0	20	,,	∞	0	20	,,	∞
	20	1	,,	5	20	1	,,	6
	20	5	,,	2	20	5	,,	50
	20	10	,,	17	20	10	,,	175
	20	20	,,	540	20	20	,,	6,000
	20	40	,,	6,000	20	40	,,	36,000
	20	60	,,	42,000	20	60	,,	unc.
	20	80	,,	unc.	20	80	,,	∞
	20	100	,,	∞	20	100	,,	∞

unc. = uncountable: ∞ = infinite.

Observations were also made with antistreptococcic immune sera, these were the sera of two horses immunised by Professor Tavel in Berne, and are denominated as S.1. and S.2. I had tested them when fresh as regards bacteriolytic power for *Streptococci* with the results

[1] 2 to 4 months old for the rabbits' antityphoid sera; and about 1 year old for the horse's antityphoid serum.

Table IX.

Showing the bacteriolytic power of the horses' antistreptococcic sera S.1. *and* S.2. *when fresh compared with that of the normal rabbits' serum* N.4.

Age of serum	Drops of serum	Streptococcus culture	Serum N.4. Colonies in plates	Serum S.1. Colonies in plates	Serum S. 2. colonies in plates
1 hour	5	1 loopful	∞	—	50,000
	10	,,	∞	—	16,000
3 hours	5	,,	∞	1,800	—
	10	,,	∞	900	—
4 ,,	5	,,	∞	—	10,000
	10	,,	∞	—	6,000
6 ,,	5	,,	∞	40,000	—
	10	,,	∞	16,000	—
24 ,,	5	,,	∞	unc.	unc.
	10	,,	∞	50,000	40,000
2 days	5	,,	∞	∞	∞
	10	,,	∞	unc.	unc.
3 ,,	5	,,	∞	∞	∞
	10	,,	∞	∞	∞

unc. = uncountable : ∞ = infinite.

exhibited in Table IX. They were already four months old when used for the observations given in Tables X. and XI.

Table X.

Showing the effect on bacteriolysis of the addition to fresh rabbits' serum of increasing quantities of the now inactive immune serum S.1. *of a horse.*

Rabbits' serum, 4 hours old. Drops	Inactive horse's antistreptococcus serum S.1. Drops	Streptococcus culture	Colonies in plates
15	0	1 loopful	32,400
15	1	,,	15,600
15	5	,,	6,500
15	25	,,	13,200
15	50	,,	54,000
15	100	,,	∞

∞ = infinite.

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TABLE XI.

Showing the effect upon bacteriolysis of the addition to the fresh sera of sheep 1 *and* 2 *of increasing quantities of the now inactive horses' immune sera* S.1. *and* S.2. *respectively.*

Fresh serum of sheep 1, 4 hours old. Drops	Inactive horses' antistreptococcus serum S.1. Drops	Strepto-coccus culture	Colonies in plates	Fresh serum of sheep 2, 4 hours old. Drops	Inactive horses' antistreptococcus serum S.2. Drops	Strepto-coccus culture	Colonies in plates
20	0	1 loopful	43,200	20	0	1 loopful	unc.
0	20	,,	∞	0	20	,,	∞
20	1	,,	13,500	20	1	,,	unc.
20	5	,,	8,100	20	5	,,	32,400
20	10	,,	16,300	20	10	,,	14,000
20	20	,,	24,300	20	20	,,	34,000
20	40	,,	35,100	20	40	,,	100,000
20	60	,,	81,000	20	60	,,	unc.
20	80	,,	unc.	20	80	,,	∞
20	100	,,	∞	20	100	,,	∞
20	150	,,	∞	20	150	,,	∞
20	200	,,	∞	20	200	,,	∞

unc. = uncountable: ∞ = infinite.

The remarkable diminution of bacteriolytic power occasioned by the presence of an excess of immune serum is shown in each of the Tables VI. VII. VIII. X. and XI. where the effect of the addition of increasing quantities of immune serum is seen to be at first a marked increase, but later when the amounts become excessive a progressive loss of the bacteriolytic action of the normal serum. An explanation of this phenomenon can be suggested, as was pointed out by Neisser, which is compatible with the views of Ehrlich on the mechanism of the bacteriolytic process, while no such explanation can be found consonant with the various other theories of the nature of bacteriolysis which are at present held by different writers. This explanation may be briefly stated thus.

Complement and immune body possess a certain mutual affinity which may conceivably be increased or lessened by a variety of influences. The attachment of the immune body to a bacterium is such an influence. If then bacteria be brought into a fluid containing their specific immune body and also complement, the former as we know becomes attached to the bacteria, and its affinity for the complement may

thus be modified. The case presents three possible alternatives as follows. The attachment of the immune body to bacteria may (*a*) increase its affinity for the complement; it may (*b*) cause no alteration at all in this affinity; or it may (*c*) diminish the affinity for complement.

If the first of these alternatives held good, no serious consequence could attend the presence of excess of immune body. For that part of it which had attached itself to the bacteria, would, by its heightened affinity for complement, be enabled to secure that substance by its complement-haptophore and the destruction of the bacteria would follow. But if the second, and much more if the third alternative holds good the free excess of immune body present will have either (1) as great, or (2) an even greater affinity for complement than that which is already attached to the bacteria. It follows, therefore, that if the third alternative correctly represents the situation, or if the second does so and a great excess of immune body be at hand, practically the whole available complement will be secured by the free immune body, and none, or very little, by the immune body already fixed in the bacteria. Bacteriolysis will therefore fail from lack of complement. This is a possible explanation of the situation in the experiments recorded. But I am not convinced that it is altogether satisfactory. Probably other factors still require investigation. The facts observed, however, appear to be unquestionable.

These observations help to explain the experience of Pfeiffer and others who have found that highly immunised animals may at times succumb to moderate multiples of the M. L. D.[1] of the bacterium against which they have been immunised; and certainly suggest the necessity of caution in the administration of large quantities of anti-microbic sera for therapeutic purposes in the human subject. And it would appear to be at least an equally important object, failing the eventual discovery of some means of introducing an additional supply of complement from external sources, to *secure the presence of adequate leucocytosis* in all conditions of infective origin, and more especially *whenever the injection of anti-microbic serum is contemplated.*

I draw the following conclusions from the observations which have been here recorded.

1. The amount of complement present in a given serum varies continuously from hour to hour after the blood is shed. It undergoes a steady increase during the first few hours if the serum be left in

[1] M. L. D. = Minimal lethal dose.

contact with the clot, and only subsequently begins to show progressive diminution. Serum removed from the clot-containing vessel and whipped blood, on the other hand, show no such increase of their complement, which undergoes a steady diminution from the first.

2. Complement (addiment) is a leucocytic product only appearing in blood-plasma or serum as the result of a disintegration of leucocytes.

3. Observations on the bacteriolytic action of a serum are only comparable *if performed with the same serum and at the same time.*

4. The exhibition of excess of immune-serum may be as harmful in the course of an infection as its entire omission; and great excess of immune-serum might perhaps directly bring about a fatal issue by absorbing all the complement and thus arresting normal protective processes.

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