ON A DYSENTERY TOXIN AND ANTITOXIN.

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CONTENTS.

I. *Introduction.*

One of the most striking points in the pathology of Bacterial Dysentery is the fact that while the bacilli are found regularly in the mesenteric glands, they are not found in the spleen or other organs, and

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do not occur in the blood, urine, or milk. In this respect Lentz (p. 320) points out that the disease differs markedly from the septicaemic diseases, such as typhoid, and that it must be regarded as a local infection of the intestinal mucous membrane and corresponding lymphatic glands by the bacillus, the toxin alone passing into the circulation and giving rise to the typical clinical picture, which, as in the case of cholera, gives the impression of a severe poisoning or toxaemia.

The results of experiments on animals with the *Bacillus dysenteriae* (Shiga) bear out this view, as the pathological changes which result from the injection of the living bacillus may be produced equally well by the use of dead cultures; showing that these changes are brought about by some toxic body, and not by any vital action of the bacillus.

The following experiments, which were commenced at the end of 1902, were undertaken with the view of investigating the nature of this toxic body, and in the hope of obtaining an anti-body for it. A preliminary note of the earlier results was published by me in the *British Medical Journal* in December, 1903. At the time the experiments were begun the only reference which could be found in the literature to a soluble dysentery toxin was the statement by Lentz (p. 328) in the article on dysentery in Kolle and Wasserman's *Handbuch der pathogenen Microorganismen*, "that the filtrates of dysentery cultures are toxic." Subsequently, however, Gay (xi. 1902) showed that vaccines prepared by killing the dysentery bacillus by heat and tricresol, or by tricresol alone, undergo on keeping an increase in toxicity accompanied by a shortening of the time necessary to produce death. This is attributed to a process of breaking up of the bacterial bodies.

Conradi (1903) also stated that he was able by a process of "aseptic autolysis" to extract from the dysentery bacillus a soluble toxin, and shortly after this Neisser and Shiga were able to obtain a similar toxin by suspending the bacillus in saline, killing by exposure to heat, keeping the suspension for two days at 37° C., and then filtering. Finally Rosenthal also found that by growing the bacillus in Martin's alkaline broth highly toxic filtrates are formed.

In the present experiments the following bacilli were used:

1. *B. dysenteriae* Shiga.
2. „ Kruse (obtained from Král).
3. „ Flexner (obtained from Král).
4. „ Flexner (Adult Dysentery, Philippines).

5. *B. dysenteriae* Duval (Summer Diarrhoea, Baltimore).
6. „ Duval (Summer Diarrhoea, New York).
7. „ Eyre No. 1 (Asylum Dysentery, England).
8. „ Eyre No. 2 „ „
9. „ Eyre No. 3 „ „

These cultures, with the exception of Nos. 2 and 3, which were obtained from Král of Prague, were very kindly sent me by Professor Flexner and Drs Shiga, Duval, and Eyre.

The toxigenic power of each of these strains was tried, but for most of the experiments Kruse's bacillus was employed, and where the strain of the bacillus is not specified this bacillus is referred to.

II. *Production and Characters of the Toxin.*

The filtrates from ordinary broth cultures of the bacillus are only slightly toxic for animals, but it was found that by using a more alkaline medium much more highly toxic filtrates could be obtained.

For the production of the toxin the ordinary alkaline broth as employed for the production of diphtheria toxin (that is, broth which has been made just alkaline to litmus and then has had added to it 7 c.c. of normal caustic soda per litre) was planted with the bacillus; grown for a varying period at 36° C., and then filtered through a Pasteur-Chamberland filter and the filtrate tested on rabbits by intravenous injection. At first the cultures were grown for ten days before filtration. The following table shows some of the results:

TABLE I. *Toxins obtained by growing the Bacillus for Ten Days in Alkaline Broth.*

No. of toxin	Bacillus	M.L.D. for large rabbits
No. 1	Kruse	1 c.c.*
„ 2	„	„
„ 3	„	„
„ 4	„	„
„ 5	„	„
„ 6	Shiga	„

* In the tests the minimal lethal dose (M.L.D.) was only approximately determined, that is, the toxin killed at 1 c.c. and not at 0·5 c.c.

Later it was found that by growing the cultures for a longer period much more toxic filtrates resulted, as shown in the following:

TABLE II.

No. of toxin	Bacillus	M.L.D. for large rabbits
K. 8	Kruse	0·10 c.c.
K. 9	,,	0·10 ,,
K. 11	,,	0·10 ,,
K. 15	,,	0·10 ,,

The best results, as regards the toxicity of the filtrates, appears to be reached in about a month to six weeks, and after this the toxicity begins to fall. The filtrate from a three months' old culture was found to be incapable of killing large rabbits at 0·5 c.c. though this dose gave rise to paralysis and diarrhoea. Experiments were made with a bouillon containing twice the amount of alkali but this did not give any increase of toxicity. The toxin in the filtrate is moderately stable—a toxin which had been kept at room temperature for 4½ months being still highly toxic—and is not destroyed by heating at 70° C. for 1 hour, though exposure to 80° C. for an hour seems to entirely destroy it. It is precipitated by ammonium sulphate; thus a filtrate which killed rabbits at 0·1 c.c. intravenously was precipitated with ammonium sulphate, the precipitate dissolved in distilled water and again precipitated. After drying *in vacuo* over sulphuric acid the final precipitate killed large rabbits at 0·002 grammes as shown in the following table:

TABLE III. *Precipitated Toxin* (*K.* 12) *tested on Rabbits intravenously.*

Dose in grammes	Weight of rabbit in grammes	Result
0·10	1050	Dead (24 hours)
0·01	880	Dead (3 days)
0·004	1250	Dead (2 days)
0·002	1480	Dead (4 days)
0·002	1700	Recovered after paralysis
0·001	1600	Lived, no paralysis
0·001	1600	Lived, slight paralysis

The results of the intravenous injection of a lethal dose of the toxin in a rabbit are practically the same as when the living, or dead bacillus is used. After a latent period, varying from one to three or four days, according to the amount of toxin injected, severe diarrhoea sets in with paralysis of the limbs, beginning sometimes in the fore, and sometimes in the hind limbs, but ultimately affecting both, as well as the muscles of the trunk and neck. The animal rapidly loses weight, and death occurs in from one to four or five days. *Post mortem* the lesions

resemble those following the injection of the bacillus. There is marked congestion of the large intestine, which may show small haemorrhages and contains a slimy fluid. The lungs show patches of congestion and often small haemorrhages, the other viscera do not, as a rule, exhibit any marked naked-eye changes.

In the rabbit the toxin acts most energetically when given intravenously, but the same result follows subcutaneous, intramuscular or intraperitoneal injections if large doses are employed.

The variation in the susceptibility of different animals to the toxin is very striking. The rabbit and horse are highly sensitive, the latter particularly so, while the guinea-pig, rat, mouse and monkey are hardly affected; thus, 0·1 g. of the precipitated toxin K. 12 (v. Table III), *i.e.*, 50 minimal lethal doses for a large rabbit, when injected subcutaneously in a guinea-pig caused no symptoms beyond transient local swelling and loss of weight.

III. *Immunisation of Animals.*

Attempts to produce an antitoxin by immunisation of rabbits were entirely negative, the serum of an animal which had received a considerable amount of toxin and which agglutinated the bacillus at 1 in 100 showing absolutely no antitoxic power. A goat was immunised with increasing doses of the toxin, beginning with 0·1 c.c. and rising to 200 c.c. The serum of this animal, taken on the 13th day after the last injection, was tested against the toxin on rabbits and showed distinct, though slight, antitoxic power. The animal was bled again on the 33rd day after the last injection and the serum from this bleeding was found to be much more highly antitoxic. This result possibly explained the want of success in the case of the rabbit when the bleedings had been carried out on the 9th day after the last injection and probably before the antitoxic power had reached its maximum. A horse was then immunised with the toxin. The details of this immunisation are interesting as showing the great susceptibility of this animal to the toxin. The toxin used was a very weak one, 1 c.c. being required to kill a rabbit when given intravenously.

The immunisation was begun in December, 1902, by giving 0·5 c.c. of this toxin subcutaneously and injections of increasing quantities were made every 3rd day for a period of 6 weeks when a dose of 150 c.c. was reached. After a dose of 2 c.c. of the toxin the horse had a rise of temperature (102·2° F.) the same evening, and the next day had very pronounced diarrhoea, and was off his feed. Later on, after a dose of

8 c.c., diarrhoea again appeared, but with these exceptions the animal appeared to stand the immunisation well. On the 5th day after the final injection of 150 c.c., however, complete paralysis of the hind legs occurred and the animal had to be killed.

A second horse was then immunised, beginning with a dose of 0·1 c.c. of a toxin of the same strength and very cautiously increasing the doses, which were repeated at intervals of not less than a week. In this case the immunisation was quite satisfactory, no symptoms being shown beyond the transient local swelling and rise of temperature following the injection. Finally, a dose of 400 c.c. of a toxin which killed a large rabbit at 0·1 c.c. was reached, after which the animal was allowed to rest for a month and then bled.

For purposes of comparison a third horse was immunised, and in this case the bacillus alone, *i.e.* without its soluble toxic products, was used. Kruse's bacillus was grown for twenty hours on neutral agar and the growth emulsified with saline. At the beginning of the immunisation this emulsion was heated for half-an-hour at 70° C., and given subcutaneously, beginning with a dose corresponding to one-tenth of 1 tube and gradually rising to 32 tubes. After this an emulsion of the living bacillus was given intravenously until a dose of 10 agar tubes of living culture was reached. One month after the last injection the horse was bled.

The subcutaneous injections did not cause any abscess formation though there was considerable swelling, the intravenous injections caused much more trouble, and after the final dose of 10 tubes of the living culture the animal was very sick, dunging and staling repeatedly with the anus patent and exuding fluid. These symptoms, however, passed off in a couple of days.

IV. *Action of the Immune Serum.*

On comparing the action of the serum of the horse (No. 2) with that of a normal horse by mixing the respective sera with the toxin and injecting the mixture intravenously in rabbits it was at once evident that while normal serum has practically no antitoxic action the immune serum is highly antitoxic. This is shown in the following table (Table IV) which gives the results of one of a large series of tests which have been carried out with this serum. In carrying out the test 1 c.c. of a toxin, the minimal lethal dose of which for a half-grown rabbit was one-fourth of a cubic centimetre (*i.e.*, about 4 M.L.D.), was mixed with varying

quantities of the serum, the mixture made up in each case to 3 c.c. and after standing for half-an-hour in the incubator at 36° C., injected intravenously in rabbits. In the earlier experiments control tests were always made with normal horse serum but as it was found that this had no appreciable protective action these were subsequently omitted.

TABLE IV. *Testing Antitoxic Power of Serum of Horse immunised with Dysentery Toxin.*

1 c.c. Toxin = 4 M.L.D.

No. of rabbit	Weight of rabbit in grammes	Toxin	Serum	Result
1.	2080	0·15 c.c.	—	Lived, after severe paralysis
2.	1930	0·20 ,,	—	Dead (8 days), after severe paralysis and diarrhoea
3.	2010	0·25 ,,	—	Dead (2 days)
4.	2915	1·0 ,,	0·02 c.c.	Lived, no symptoms
5.	2380	1·0 ,,	0·01 ,,	,, ,,
6.	1375	1·0 ,,	0·005 ,,	,, ,,
7.	1835	1·0 ,,	0·0025 ,,	,, ,,
8.	1775	1·0 ,,	0·0014 ,,	,, ,,
9.	1245	1·0 ,,	0·0010 ,,	,, ,,

TABLE V. *Testing Antitoxic Power of Serum of Horse immunised with Toxin (Kruse).*

1 c.c. Toxin = 20 M.L.D.

No. of rabbit	Weight of rabbit in grammes	Toxin	Serum	Result
1.	560	1 c.c.	0·005 c.c.	Lived, no symptoms
2.	620	1 ,,	0·002 ,,	Lived, no symptoms
3.	630	1 ,,	0·001 ,,	Dead (2 days)
4.	690	1 ,,	0·0005 ,,	Dead (2 days)

As the limits of the protective power were not reached in the first series of experiments (Table IV) a further test was made with a much stronger toxin. The result of this is shown in Table V, where it will be seen that the serum has a very powerful antitoxic action, one five-hundredth of a cubic centimetre being sufficient to neutralise 20 minimal lethal doses of the toxin when the two are mixed and kept together for a time before injection.

The prophylactic action of the serum is particularly interesting. This is shown in Tables VI and VII. It will be seen that if the serum be given intravenously it protects absolutely against large doses of the toxin given half-an-hour later. If, however, the serum be given 24 hours before the toxin it does not protect or shows only very slight

protection. This may be due to the excretion or destruction of the antitoxin during the intervening 24 hours. If the toxin be given intravenously in one ear the animals can be saved by an injection of the serum into the other ear five minutes later.

TABLE VI. *Prophylactic Action of Immune Serum.* (*Serum given intravenously in right ear; toxin* (*K.* 8) *given intravenously in left ear half-an-hour later.*)

No. of rabbit	Weight of rabbit in grammes	Serum	Toxin (K. 8)	Result
		Immune		
1.	1345	4·0 c.c.	0·5 c.c.	Lived, no symptoms
2.	1655	4·0 ,,	1·0 ,,	,, ,,
3.	1705	4·0 ,,	2·0 ,,	,, ,,
4.	1710	4·0 ,,	2·0 ,,	,, ,,
5.	1705	4·0 ,,	2·0 ,,	,, ,,
6.	1715	4·0 ,,	4·0 ,,	,, ,,
		Control		
7.	1670	4·0 ,, normal	0·5 ,,	Dead (20 hours)

TABLE VII. *Prophylactic Action of Immune Serum.* (*Serum given intravenously in right ear; toxin* (*K.* 8) *given intravenously in left ear 24 hours later.*)

No. of rabbit	Weight of rabbit in grammes	Serum	Toxin	Result
		Immune		
1.	1635	4·0 c.c.	2·0 c.c.	Lived, transient paralysis in forelegs
2.	1370	4·0 ,,	2·0 ,,	Dead (4 days) after paralysis and diarrhoea
3.	1555	4·0 ,,	2·0 ,,	Lived, transient paralysis in forelegs
4.	1650	4·0 ,,	4·0 ,,	Dead (2 days)
		Controls		
5.	1970	4·0 ,, normal	0·5 ,,	Dead (40 hours)
6.	1695	4·0 ,,	0·5 ,,	Dead (20 hours)
7.	1470	4·0 ,,	0·5 ,,	Dead (20 hours)
8.	1740	4·0 ,,	1·0 ,,	Dead (40 hours)

In the earlier experiments with the antitoxic serum certain irregularities were observed in its action, a given amount of serum sometimes protecting an animal completely and at other times, though saving the animal, failing to completely avert the paralysis and diarrhoea. This was subsequently found to be due to the fact that the toxin and antitoxin require a certain time for their combination. Thus, when 3 c.c. of toxin were mixed with 0·5 c.c. of a somewhat weak serum and

the mixture injected immediately into the ear vein of a rabbit the animal died in four days after severe paralysis and diarrhoea. When the same mixture was kept in the incubator for an hour before injection the animal showed no symptoms. In order to determine the action of temperature on the time of combination two parallel series of experiments were made. In these the toxin and serum were brought to the desired temperature, mixed and kept at this temperature, for varying times, after which they were injected intravenously into rabbits. The results are shown in Tables VIII and IX.

TABLE VIII. *Showing time necessary for combination of Toxin and Antitoxin at 37° C.*

No. of rabbit	Weight of rabbit in grammes	Serum	Toxin	Serum and toxin mixed and injected after	Result
1.	1805	0·5 c.c.	3 c.c.	Immediately	Recovered after severe paralysis
2.	1725	0·5 ,,	3 ,,	5 minutes	No symptoms
3.	1525	0·5 ,,	3 ,,	15 ,,	,,
4.	1500	0·5 ,,	3 ,,	60 ,,	,,

TABLE IX. *Showing time necessary for combination of Toxin and Antitoxin at 0° C.*

No. of rabbit	Weight of rabbit in grammes	Serum	Toxin	Serum and toxin mixed and injected after	Result
1.	1550	0·5 c.c.	3·0 c.c.	4 minutes	Dead (32 hours)
2.	1480	0·5 ,,	3·0 ,,	8 ,,	Dead (42 hours)
3.	1700	0·5 ,,	3·0 ,,	15 ,,	Dead (3 days)
4.	1280	0·5 ,,	3·0 ,,	30 ,,	,,
5.	1300	0·5 ,,	3·0 ,,	60 ,,	,,
6.	1640	0·5 ,,	3·0 ,,	2 hours	Lived, no symptoms
7.	1250	0·5 ,,	3·0 ,,	5 ,,	,, ,,
8.	990	0·5 ,,	3·0 ,,	10 ,,	,, ,,
9.	1655	0·5 ,,	3·0 ,,	20 ,,	,, ,,

From these it is seen that whereas at 37° C. the combination of toxin and antitoxin is complete in less than five minutes; at 0° C. this combination requires a time of between one and two hours.

The fact that the action of dysentery toxin is not abolished by the presence of an equivalent amount of the antitoxin if the mixture is injected immediately on mixing is due to (1) the toxin and antitoxin requiring a certain time for their combination and (2) the free toxin becoming very rapidly fixed by the tissues.

Both these facts are in accordance with our experience in the case of other toxins. The credit of first drawing attention to the conditions

of neutralisation, in respect both of time and of amount of toxin and antitoxin, belongs to Fraser (1896), who investigated these points in the case of venom and antivenin. These conditions were further investigated by Martin and Cherry (1898) for venom and diphtheria toxin, by Ehrlich (1897) and by Knorr (1897) for tetanus toxin.

The rapid fixation of dysentery toxin by the tissues of the body is entirely similar to the fixation of tetanus toxin as pointed out by Dönitz (1897).

The antitoxin is very stable and is not destroyed by exposure to a temperature of 65°—66° C. for 1 hour.

From the foregoing it is evident that old cultures of the dysentery bacillus contain a soluble toxic body which is capable of giving rise in the horse to a powerful antitoxin, and the question naturally arises as to whether this toxic substance is to be regarded as a soluble toxin secreted by the bacillus into the surrounding fluid as in the case of diphtheria and tetanus or is it rather to be looked upon as more properly an intracellular toxin which has soaked out of the cell. There appears to be a large balance of evidence in support of the latter view, thus old cultures (4—6 weeks) which must contain a large number of dead and macerated bacilli are much more toxic than younger cultures (8 to 12 days) when more of the bacilli are alive. This is the reverse of what obtains in the case of diphtheria and tetanus.

Again, by autolysis of quite young cultures of dysentery highly toxic filtrates can be obtained, as was shown by Conradi (1903) and also Neisser and Shiga (1903). In order to ascertain if the toxin obtained by the autolysis of young cultures is the same as that found in solution in old alkaline broth cultures a toxin was prepared from a young agar culture by Neisser and Shiga's method. It was found that 1 c.c. of this (*i.e.*, 20 minimal lethal doses) was completely neutralised by 1 c.c. of the serum of horse No. 2, which had been immunised with the filtrates from old alkaline broth cultures, showing that the toxin is the same whether obtained from the autolysis of quite young cultures or from the growth of the bacillus for some weeks in alkaline broth. Further evidence in the same direction was obtained from the immunisation of horse No. 3, which had received only young agar cultures. The serum of this animal was tested against a toxin obtained in the usual manner by growing the bacillus in alkaline broth. The result is shown in Table X, from which it will be seen that this serum was more powerfully antitoxic than that of horse No. 2, which had received large doses of the toxin (cf. Table IV), one-thousandth of a cubic centimetre of the

serum completely neutralising twenty minimal lethal doses of the toxin.

TABLE X. *Testing Antitoxic Power of Serum of Horse immunised with Kruse's bacillus.*

1 c.c. Toxin = 20 M.L.D.

No. of rabbit	Weight of rabbit in grammes	Toxin	Serum	Result
1.	870	1·0 c.c.	0·1 c.c.	Lived, no symptoms
2.	670	1·0 ,,	0·03 ,,	,, ,,
3.	750	1·0 ,,	0·01	,, ,,
4.	650	1·0 ,,	0·003 ,,	,, ,,
5.	680	1·0 ,,	0·001 ,,	,, ,,
6.	610	1·0 ,,	0·0005 ,,	Died (2 days)
7.	570	1·0 ,,	0·0002 ,,	,,

If the dysentery bacillus is grown for 24 hours on neutral agar and the growth emulsified in saline and killed by exposure to chloroform vapour for an hour the emulsion of the dead bacilli is highly toxic for rabbits, killing them with the same symptoms as the living bacillus.

A large series of experiments was carried out in order to ascertain if the serum of horse No. 2 (immunised with the toxin only) was capable of protecting rabbits against the dead bacillus. The serum and suspension were given as follows:

(1) Serum and suspension mixed and given intravenously.

(2) Serum intravenously followed after half-an-hour by the suspension also intravenously.

(3) Serum intravenously—suspension subcutaneously at the same time.

(4) Serum intravenously—suspension intraperitoneally at the same time.

(5) Serum subcutaneously—suspension intravenously the following day.

In all the above series control experiments were made with normal serum and it was found that while the control animals all died with the typical symptoms in every case, the animals receiving the antitoxic serum were entirely protected.

V. *Comparison of the toxins obtained from bacilli of various strains and the relation of these toxins to the antitoxin.*

The formation by *B. dysenteriae* Kruse of a soluble toxin which can be neutralised by antitoxin suggests an interesting method of attacking

the question of the identity, or otherwise, of the various races of bacilli which have been described in connection with epidemics of bacterial dysentery and allied diseases. With this view nine races of bacilli were examined. The comparative agglutination of these with the serum of horse No. 3 (which had been immunised with Kruse's bacillus only) is shown in Table XI, from which it is seen that the bacilli fall into two classes:— one class, more highly agglutinated by the serum, including bacilli of Shiga, Kruse, and the three strains isolated from cases of Asylum Dysentery in England by Eyre (1904), and a second class which are less highly agglutinated, consisting of Flexner's Philippine dysentery bacillus, and two races of bacilli isolated by Duval from cases of summer diarrhoea in Baltimore and New York. The action of the bacilli on mannite (Lentz, 1902, p. 559) agrees completely with the agglutination tests, as none of the members of the first class cause any alteration in the reaction of the medium, while the four less highly agglutinated races constituting the second group all cause definite formation of acid.

TABLE XI. *Agglutination of Dysentery Bacilli with Serum of Horse No.* 3 (2 *hours at* 36° *C. and then over-night at room-temperature*).

	1/100	1/200	1/500	1/1000	1/2000	1/4000
Kruse (Král)	+ + +	+ + +	+ +	+	0	0
Shiga (Shiga)	+ + +	+ + +	+ +	+	0	0
Flexner (Dysentery, Philippines)	+	+	0	0	0	0
Flexner (Král)	+ +	+	0	0	0	0
Duval (New York, Summer Diarrhoea)	+ +	+	0	0	0	0
Duval (Baltimore, Summer Diarrhoea)	+ +	+	0	0	0	0
Eyre (No. 1, Asylum Dysentery)	+ + +	+ +	+ +	+	0	0
Eyre (No. 2, Asylum Dysentery)	+ + +	+ +	+	0	0	0
Eyre (No. 3, Asylum Dysentery)	+ + +	+ + +	+ +	+	0	0

An examination of the toxigenic power showed entirely similar results. The filtrates from one month old cultures in alkaline broth in the case of Shiga's bacillus and the three Asylum dysentery bacilli were all toxic as shown below:

TABLE XII. *Toxicity of Filtrates of Alkaline Broth Cultures* (1 *month old*) *for Rabbits* (*intravenous*).

	Dose	Result
B. dysenteriae Shiga	0·2 c.c.	Died (3 days)
Asylum Dysentery Eyre (No. 1)	1·0 ,,	Died (2 days)
,, ,, (No. 2)	1·0 ,,	,,
,, ,, (No. 3)	1·0 ,,	,,

all the animals showing the typical paralysis.

The effect of these toxins could, moreover, be entirely neutralised by the antitoxic serum of horse No. 2 (which had received the toxin of Kruse's bacillus only), using from four to ten minimal lethal doses of the various toxins. On the other hand the filtrates from the American bacilli in doses of 5 c.c. intravenously did not affect the animals with the exception of causing diarrhoea, which in some cases was very marked.

TABLE XIII. *Toxicity of Filtrates of Alkaline Broth Cultures (1 month old) for Rabbits (intravenous).*

	Dose	Result
B. Flexner (Philippines)	5 c.c.	Lived, diarrhoea, no paralysis
B. Flexner (Král)	5 ,,	Lived, no diarrhoea or paralysis
B. Duval (Baltimore)	5 ,,	Lived, diarrhoea, no paralysis
B. Duval (New York)	5 ,,	Lived, no diarrhoea or paralysis

These results would appear to support the views of Martini and Lentz (1902), but, on the other hand, they might be explained by the fact that in the case of the American bacilli examined the pathogenic power may have fallen to a very low value, or that, as suggested by Gay (1903), the bacilli belonging to the two types are members of a closely related group of organisms. The latter view is supported by the fact demonstrated by Gay that the antidysenteric serum is effective in different doses for both types of the microorganism.

The formation of a very definite toxin by the bacillus isolated by Eyre from a case of Asylum dysentery in England and the fact that this toxin is neutralised by the antitoxin prepared by the use of the toxin from Kruse's bacillus is particularly interesting.

The comparative action of the sera resulting from the immunisation of horses with the toxin, and with the bacillus on the infection of rabbits with the living bacillus, as well as the comparative bactericidal action of these sera *in vitro*, is at present under investigation.

The fact that the serum of animals immunised with the dysentery bacillus possesses powerful antitoxic properties appears to have been overlooked by previous workers. Shiga in 1901 published the results of his experiments with a serum obtained by immunising animals with the dysentery bacillus, and this serum was used clinically in a large number of cases with excellent results. Kruse (1903) and Gay (1902) working under Flexner also prepared a serum in the same manner and obtained good results. Both Shiga and Kruse appear to have attributed the protective action of the immune serum to its bactericidal action,

and Shiga (1903) points out that this is the first serum used for therapeutic purposes in man which fulfils the conditions laid down by Ehrlich in the Croonian Lecture of 1900, viz., that it contains an immune body for which a suitable complement exists in human serum. Shiga regards his clinical results as supporting Ehrlich's view, but the question of the serum being antitoxic, as well as bactericidal, does not appear to have been considered.

Gay (1902) showed that his serum protected against single lethal doses of vaccine, but though in his second paper (1903) he says that bacteriolysis is not an index of the protective power *in vivo* of antidysenteric serum he makes no statement as to the important antitoxic properties possessed by this serum[1].

CONCLUSIONS.

1. Old cultures of *B. dysenteriae* Kruse in somewhat highly alkaline broth contain a soluble toxin.

2. The same toxin is also contained in the bodies of the young bacilli.

3. The horse and rabbit are very highly susceptible to this toxin, the guinea-pig, rat, and mouse being very resistant.

4. Immunisation of the horse, either with the soluble toxin from old alkaline broth cultures, or with the bodies of the young bacillus, gives rise to an antitoxin.

5. The antitoxic power of the serum of horses so immunised may reach a very high value—in the case of an animal immunised with the bacillus one-thousandth of a cubic centimetre of the serum being sufficient to protect a small rabbit against twenty minimal lethal doses of the toxin.

6. The antitoxin is capable of protecting animals, either when mixed with the toxin, or when given separately at another part of the body either at the same time or shortly before or after the toxin.

7. The toxin and antitoxin require a certain time for their combination *in vitro*, and this time is dependent upon the temperature,

[1] *Note during preparation.* In a paper published quite recently Rosenthal (1904) describes a serum which he has obtained by injecting horses with both the toxin and cultures; this serum possesses both antitoxic and bactericidal properties, and he has been able to use it in the treatment of 157 cases of bacterial dysentery with most encouraging results.

varying from less than 5 minutes at 37° C., to between one and two hours at 0° C.

8. Shiga's Dysentery bacillus and three strains of a bacillus isolated from cases of Asylum Dysentery in England by Eyre were found to yield a similar toxin, and this toxin was neutralised by the antitoxin prepared by means of the toxin from Kruse's bacillus. A strong point in favour of the identity of the above bacilli.

9. Attempts to obtain a soluble toxin from *B. dysenteriae* Flexner (adult dysentery, Philippines) and from two races of the bacillus isolated from cases of summer diarrhoea by Duval in Baltimore and New York were unsuccessful.

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