TOXINS AND ANTITOXINS OF BACILLUS DYSENTERIÆ SHIGA.

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PLATES 6 TO 8.

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The nature of the toxin of the Shiga dysentery bacillus has been studied by a number of bacteriologists, notably by Shiga, Neisser and Shiga, Conradi, Vaillard and Dopter, Rosenthal, Todd, Kraus and Doerr, Flexner and Sweet, Doerr, Pfeiffer, Bessau, and Lüdke. But no agreement as to its precise nature has as yet been reached.

The chief discrepancies in experimental results and in deductions arrived at from them may perhaps be due to difference in method of preparing the toxin. Those who regarded the latter as an endotoxin prepared it by washing off the growth on agar slants with saline solution, shaking, heating to 55° or 60°C., incubating for 24 to 48 hours, and filtering through a Berkefeld candle. Those, on the other hand, who viewed it as an exotoxin obtained it by growing the bacilli in alkaline broth for a period of 2 to 6 weeks and then filtering.

1 Shiga, K., Centr. Bakteriol., 1te Abt., 1898, xxiii, 599.
9 Doerr, R., Das Dysenterietoxin, Jena, 1907, 30 ff.
10 Pfeiffer, Centr. Bakteriol., 1te Abt., Ref., Beilage, 1908, xlii, 1.
12 Lüdke, H., Die Bazillenruhr, Jena, 1911, 90.
Our studies of the toxic products yielded by the Shiga bacillus have led us to the conclusion that this microorganism growing in vitro produces two poisons, one an endotoxin, the other an exotoxin, which can be separated experimentally and can also be shown to attack different anatomical structures of the rabbit and to set up two distinct kinds of pathologic effects.

Shiga first pointed out that the bacillus which bears his name is highly toxic for the rabbit, and this animal has remained the chief one for demonstrating experimentally the pathogenic action of the microorganism. The Shiga bacillus or its poisonous products induces two kinds of marked lesions in the rabbit; one is localized in the intestine, and the other in the central nervous system.

The first comprehensive study of the lesions in the central nervous system was made by Dopter. He concluded that the central nervous system is usually the seat of serious lesions which may occur in any portion of the system, although the medulla is most often affected. The gray matter, and almost exclusively the anterior horns, show chromatolysis of the neurons in a varying degree and, besides, at times, areas of necrosis which destroy the cellular elements and myelin fibers, leaving scarcely any vestiges of them. At the same time there are an intense hyperemia and even hemorrhages invading the tissue. The white matter is intact. In short, the lesion is that of an acute myelitis, often an anterior poliomyelitis, and sometimes a polioencephalitis as well.

The intestinal lesions were studied by Flexner and Sweet who state that they vary in intensity. The coats of the large intestine are greatly thickened by inflammatory edema, in which case the mucosa is yellowish white and thrown into deep folds and corrugations, or more or less hemorrhage may be associated with the edema. At another time the transverse folds of mucous membrane are affected chiefly; they are swollen, the edges are hemorrhagic, and a pseudomembrane is scattered over the surface. Or, again, the transverse folds are greatly affected and the intervening mucosa is less affected, while patches of swollen and hemorrhagic mucous membrane, covered with a false membrane, appear upon and between the folds. The hemorrhage may extend into the serous coat.

The Exotoxin of Bacillus dysenteriae Shiga.

Preparation of the Exotoxin.—Comparative studies were made of media favoring a high yield of exotoxin as well as the conditions influencing its production. The following protocols are illustrative.

A. Yield of Toxin in Plain Broth and Egg Albumin Broth. A quantity of plain meat infusion broth was divided into two lots. To the first was added one-third of its volume of a 10 per cent egg albumin solution. Both lots were adjusted to a pH of 7.8 and were inoculated with the same amount of a 24 hour broth culture of Shiga bacilli, Strain 114 S. After incubating for 5 days the cultures were filtered through a Berkefeld N candle and tested on rabbits weighing 1,500 gm. Table I shows the results.

TABLE I.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Rabbit No.</th>
<th>Amount Inoculated Intravenously</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain broth</td>
<td>1</td>
<td>1.0</td>
<td>Paralysis of posterior extremities in 24 hrs.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.5</td>
<td>Died in 36 hrs. No intestinal lesions.</td>
</tr>
<tr>
<td>Egg albumin broth</td>
<td>3</td>
<td>1.0</td>
<td>Paralysis of anterior extremities in 18 hrs.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.5</td>
<td>Paralysis of both extremities in 36 hrs.</td>
</tr>
</tbody>
</table>

B. Yield of Toxin in Media with Different Degrees of Aeration.—500 cc. of egg albumin broth were placed in a 2 liter flask giving the medium a surface diameter of 17 cm. and a depth of 2 cm. An equal amount was placed in a 500 cc. flask giving the medium a surface diameter of 7 cm. and a depth of 9 cm. Both lots were inoculated with Shiga bacilli, Strain 109, incubated for 7 days, filtered, and tested (Table II).

TABLE II.

<table>
<thead>
<tr>
<th>Condition of medium</th>
<th>Rabbit No.</th>
<th>Amount Inoculated Intravenously</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep</td>
<td>5</td>
<td>0.05</td>
<td>No effect.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.10</td>
<td>Paralysis in 4 days.</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.50</td>
<td>&quot; 2 &quot;</td>
</tr>
<tr>
<td>Shallow</td>
<td>8</td>
<td>0.01</td>
<td>No effect.</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.05</td>
<td>Paralysis in 1 day.</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.10</td>
<td>&quot; 2 days.</td>
</tr>
</tbody>
</table>

14 It is to be understood where a single protocol only is given that all the experiments were repeated one or more times.
C. Relation of the Reaction of the Medium to the Yield of Toxin.—In order to determine the relation of the reaction of the medium to toxin production, small amounts of the culture fluid were tested daily with respect to reaction change and toxic potency.

Lot 15 of egg albumin broth, with an initial reaction of pH 7.6, was inoculated with Shiga bacilli and incubated at 37°C. The results are shown in Table III.

Text-Fig. 1. Relation of the pH of the medium to exotoxin production. Broth rendered sugar-free.

It is evident that the changes in the reaction of the medium, the development, in itself, of acid, do not influence the toxicity. But, as is shown in Text-figs. 1 and 2, no toxin is produced in the acid phase; it appears at the beginning of the alkaline phase, and increases thereafter.
TABLE III.

Relation of the Reaction of the Medium to the Yield of Toxin.

<table>
<thead>
<tr>
<th>Length of incubation</th>
<th>Reaction</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr.</td>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.6</td>
<td>None.</td>
</tr>
<tr>
<td>24</td>
<td>7.2</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>48</td>
<td>7.2</td>
<td>&quot; Minimum lethal dose, 5 cc.</td>
</tr>
<tr>
<td>72</td>
<td>7.3</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>96</td>
<td>7.4</td>
<td>&quot; Minimum lethal dose, 1 cc.</td>
</tr>
<tr>
<td>120</td>
<td>7.4</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>7 days</td>
<td>7.6</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

TEXT-Fig. 2. Relation of the pH of the medium to exotoxin production.
Broth not rendered sugar-free.
D. Relation of the Muscle Sugar Content of the Medium to the Yield of Toxin.—Lot 14, egg albumin broth, was not rendered sugar-free but otherwise was treated like Lot 15. There was no striking difference in the toxicity, as revealed by comparing Text-figs. 1 and 2. The removal of the muscle sugar does not influence the toxicity.

On the basis of these experiments the procedure adopted for the production of exotoxin was as follows:

Plain meat infusion broth was mixed with one-third its volume of a 10 per cent solution of egg albumin. The latter was prepared by adding one volume of the whites of eggs to nine volumes of distilled water. The mixture was adjusted to pH = 7.6 to 7.8 and quantities of 500 cc. were distributed into 2 liter flasks to permit sufficient aeration and autoclaved for 45 minutes at a pressure of 15 pounds.

This medium was inoculated with one-half an agar slant of a 24 hour culture of the Shiga bacillus and incubated at 37°C. During the period of incubation the contents of the flasks were thoroughly shaken from time to time in order to increase aeration. At the end of 5 days the culture fluid was filtered through a Berkefeld N candle. The filtrate, if proved free from bacteria, constituted the exotoxin.

Nature of the Exotoxin.

Pathologic Effects.—The rabbit is very susceptible and reacts regularly to the action of the exotoxin, the effect depending on the amount injected.

A sublethal dose injected intravenously results in the development of paresis or paralysis of the extremities within 2 to 4 days. Both anterior and posterior extremities may be affected; the former are more frequently involved. The paralytic or paretic stage may endure for 1 to 3 days and may be followed by complete or partial recovery. During this period the animal is apathetic, has no appetite, and loses weight, but intestinal symptoms are either wholly absent or inconspicuous.

A lethal dose injected intravenously results in early paralysis and prostration; that is, within 24 to 48 hours. There is considerable loss of weight. Involuntary evacuations occur but without blood or mucus. Death follows in 1 to 2 days. The autopsy findings are illustrated in the following protocol.
Rabbit 11 (Figs. 1 to 3).—Oct. 29, 1917, 2 p.m. Injected intravenously with 0.05 cc. of Exotoxin 2. Oct. 31. Paralysis of posterior extremities. Temperature subnormal. Nov. 1. Prostrated. Incontinence of urine. Stools are frequent, formed, without blood or mucus. 3 p.m. Died.

Autopsy.—There were no evident lesions in the intestines (Fig. 3) or other viscera. The cerebrospinal system, however, showed severe lesions. The meninges were free from inflammatory reaction. The gray matter, especially of the medulla and cervical cord, and only slightly of the lumbar cord, was the site of the effects, which consisted of hemorrhage, quite extensive and visible to the naked eye, and, even oftener, multiple, discrete, interstitial hemorrhages as shown in Fig. 1. Areas of necrosis were scattered throughout the gray matter. A perivascular lesion was noted. The neurons showed atrophy or even complete dissolution in limited areas. In places there was chromatolysis, granular degeneration, or caryorrhexis of the cells. The perivascular lesion consisted in an infiltration of small round cells about the arterioles and capillaries, either as a single layer about or in the sheath of the vessels, or less frequently, as a dense, heaped up infiltration, as shown in Fig. 2. The same figure shows an excess of round cells throughout the gray matter. There was a moderate edema of the gray and white matter; otherwise the white matter and nerve fibers were not affected.

The lesions in the nerve tissue are characteristic and constant and agree with those described by Dopter of the nerve injury caused by the whole dysentery toxin, except that he did not describe the perivascular lesion.

The following series of experiments was undertaken to determine whether this toxin is of the nature of an exotoxin.

Period of Incubation.—To satisfy the requirements of the class of true toxins, a poison should show a definite period of incubation before the distinctive pathologic effects develop.


The incubation period therefore depends on the dose. The period for one minimum lethal dose is usually from 24 to 48 hours, although
we have noted from fifteen similar sets of experiments that it varies actually from a few hours to 4 days.

_Globulin Fractionating._—Another point of comparison with the true toxins relates to the globulin fractionating of the poison.

**Experiment 2.**—Jan. 2, 1918. Rabbit A was injected intravenously with a globulin precipitate of Toxin 4. The globulin was purified and 0.2 cc. of a suspension in saline solution, equivalent to four minimum lethal doses, was injected. Jan. 3. Paralysis of both anterior extremities. Prostration. Loss of 70 gm. in weight. Jan. 4. Died.

_Autopsy._—No visceral lesions. Macroscopic hemorrhages in gray matter of medulla.

_Resistance to Heat._—An important difference between exotoxin and endotoxin is the thermolability of the former and the thermostability of the latter.

**Experiment 3.**—Several sets of rabbits were injected intravenously with four to ten minimum lethal doses of exotoxin which was heated for varying periods of time at temperatures from 60–90°C. As a control, endotoxin, to be described later, was submitted to similar tests. It was determined that the exotoxin was inactivated or destroyed when heated to 75°C. for 1 hour.

_Production of Antitoxin._—The toxin yields an antitoxin which will be described in detail later.

_The Law of Multiple Proportions._—The following experiment is selected from a series to show that Shiga exotoxin conforms to this law.

**Experiment 4.**—10 cc. of Toxin 4 equivalent to 100 minimum lethal doses were mixed with varying amounts of antitoxemic serum and incubated for ½ hour at 37°C. A series of rabbits was injected intravenously and it was found that 0.001 cc. neutralized one lethal unit. In other words, the antitoxic serum contained 1,000 antitoxic units.

Three other toxins were tested with the same antitoxic serum and all were neutralized in the same proportion.

_Specificity of Neutralization._—A series of control experiments to determine the effect of non-specific sera on the exotoxin shows that no neutralization is obtained.

**Experiment 5.**—Three series of tests were made with normal horse, antitetanic, and antimeningococcic serum. Two to four minimum lethal doses of the exotoxin were mixed with 1 to 5 cc. of these sera and incubated for ½ hour at 37°C. The
mixtures were then injected intravenously in rabbits. In all instances the typical neurotoxic effect of the toxin appeared.

Identity of Toxins of Different Strains.—From the standpoint of identification of the toxin as well as of specific therapy, it is desirable to know whether Shiga bacillus strains from different sources yield the same product. Exotoxins were prepared from the following strains: No. 100 from Newport News, Virginia, on artificial medium 1 year; No. 109 from Poughkeepsie, New York, 2 years; No. 114 F from Japan, more than 10 years; No. 114 S from Germany, more than 10 years; and No. 114 T, source unknown, but on artificial medium for many years. The toxins yielded by all these strains were neutralized by the antitoxic serum produced with Strain 109.

It is evident then that strains from widely different sources produce similar exotoxins and that the exotoxin production is a constant phenomenon of the Shiga bacillus, modified slightly if at all by prolonged artificial cultivation.

Table IV.

<table>
<thead>
<tr>
<th>Reaction of medium</th>
<th>Period of incubation</th>
<th>Amount of filtrate inoculated</th>
<th>Results</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.6</td>
<td>0</td>
<td>0</td>
<td>No effect.</td>
<td>Exotoxin.</td>
</tr>
<tr>
<td>7.1 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.1 2</td>
<td>5.0</td>
<td></td>
<td>No intestinal lesions.</td>
<td>Intestinal lesions.</td>
</tr>
<tr>
<td>7.2 3</td>
<td>1.0</td>
<td>Paralysis in 48 hrs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.2 4</td>
<td>1.0</td>
<td>&quot; &quot; 48 &quot; &quot; &quot; &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.3 5</td>
<td>0.5</td>
<td>&quot; &quot; 48 &quot; Died in 72 hrs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.3 7</td>
<td>0.5</td>
<td>&quot; &quot; 48 &quot; &quot; 72 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5 14</td>
<td>0.25</td>
<td>&quot; &quot; 48 &quot; &quot; 72 &quot; Intestinal lesions.</td>
<td></td>
<td>Endotoxin.</td>
</tr>
<tr>
<td>7.8 21</td>
<td>0.25</td>
<td>No paralysis. Died in 48 hrs. Marked intestinal lesions.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is evident then that strains from widely different sources produce similar exotoxins and that the exotoxin production is a constant phenomenon of the Shiga bacillus, modified slightly if at all by prolonged artificial cultivation.

Rate of Production.—The period of incubation of the culture has an important bearing on the production and nature of the toxic product. The prolongation of incubation leads, as will be shown later, to formation of endotoxin, which complicates the results. Table IV,
selected from a series of similar experiments, shows that the exotoxin develops relatively early and as incubation proceeds tends to diminish, while the endotoxin production rises.

To summarize, the Shiga bacillus grown in a favorable medium yields, in the first days of the cultivation, at the beginning of the alkaline phase of its growth, and before bacterial disintegration sets in, a toxic product which appears in the bacteria-free filtrate. This toxic product is precipitated with the globulin fraction of the protein, is relatively thermolabile, is capable of inciting antitoxin formation, is constant in properties, independently of the source of the Shiga culture, and produces in rabbits, after a definite incubation period, typical lesions of the central nervous system without at the same time, in an obvious way, injuring the intestines. In view of its peculiar properties we regard it as an exotoxin and a neurotoxin.

The Endotoxin of Bacillus dysenteriae Shiga.

Preparation of the Endotoxin.—The production of the endotoxin of Shiga bacilli does not differ essentially from that of other bacteria. The principle underlying all the methods is that of the autolysis or dissolution of the bacterial cell with the resultant liberation of its intracellular components. Most observations with the Shiga bacillus have been made with endotoxins produced in broth cultures by prolonged incubation (beyond 14 days). When a more rapid yield of endotoxin was desired, the following method was used.

Shiga bacilli were grown in Blake bottles for 24 hours. The growth was then washed off in saline solution, 15 cc. to each Blake bottle, incubated for 2 days at 37°C., and filtered through a Berkefeld N candle. We found that with Strain 100, 2.5 cc. of the filtrate prepared in this manner were lethal for rabbits weighing 1,500 to 1,800 gm.

Separation of Exotoxin from Endotoxin.—The technical difficulty of preparing pure endotoxin or exotoxin directly from the Shiga bacillus is great. Usually small amounts of one are found with the other. To establish the integrity of each of the two toxins and their independent action on the rabbit, separation of one from the other was necessary. The removal of exotoxin was accomplished by one of the methods given below.
Experiment 6. Separation by Heat.—Toxic Filtrate 16 was prepared by growing Shiga bacilli, Strain 100, in egg albumin broth for 22 days, and filtering.

Rabbit A (Control).—Injected intravenously with 1 cc. (four minimum lethal doses) of this filtrate. Paralysis of left posterior extremity after 48 hours, associated with a persistent blood-streaked mucus discharge from the intestines. Died after 4 days.

Autopsy.—Typical lesions in the medulla and intestines. Effects due to mixture of exotoxin and endotoxin.

Rabbit B.—The toxic filtrate was then heated at 80°C. for 1 hour. 1 cc. (four minimum lethal doses) was injected intravenously in Rabbit B. After 24 hours diarrhea but no nervous symptoms. Died after 4 days.

Autopsy.—Large intestine showed hemorrhagic and other lesions; cerebrospinal nervous system normal. Exotoxin destroyed by heat.

Rabbit C.—The toxin was also heated to 90°C. for 1 hour. Rabbit C was injected intravenously with 1 cc. (four minimum lethal doses). No effect. Exotoxin and endotoxin both destroyed.

TABLE V. Neutrabizagon Experiments with Various Combinations.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Exotoxin.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; + endotoxin.</td>
<td>Antixotoxic.</td>
<td>No effect.</td>
</tr>
<tr>
<td>&quot; + &quot;</td>
<td>&quot; + antibacterial.</td>
<td>Intestinal lesions.</td>
</tr>
<tr>
<td>&quot;</td>
<td>Antibacterial (containing antixotoxic).</td>
<td>No effect.</td>
</tr>
</tbody>
</table>

Experiment 7. Separation by Neutralisation.—Toxic Filtrate 18 was prepared by growing the Shiga bacilli, Strain 100, in two Blake bottles for 24 hours, washing off with a total of 30 cc. of salt solution, incubating at 37°C. for 2 days, and filtering.

Rabbit A (Control).—Injected intravenously with 2 cc. (four minimum lethal doses) of Filtrate 18. After 20 hours paralysis and intestinal symptoms. Died in 24 hours.

Autopsy.—Nervous and intestinal lesions. Mixture of exotoxin and endotoxin.

Rabbit B.—Injected intravenously with 2 cc. (four minimum lethal doses) of Filtrate 18 to which 1 cc. of antixotoxic serum had been added previous to incubation for ½ hour at 37°C. After 24 hours severe diarrhea and prostration. No nervous symptoms. Died after 3 days.

Autopsy.—Intestinal but no nerve tissue lesions. Neutralization of exotoxin by antixotoxic serum.

Other combinations were tested by injection into rabbits as shown in Table V.
BACILLUS DYSENTERIE SHIGA

Nature of the Endotoxin.

**Pathologic Effects.**—Rabbits are as uniformly susceptible to the effects of the endotoxin as they are to those of the exotoxin. If a sublethal dose is injected intravenously the rabbit shows, after 24 to 48 hours, subnormal temperature, loss of weight, and diarrhea. The stools are frequent and mucoid, occasionally blood-tinged. This condition, during which no nervous symptoms are noted, endures for 2 to 3 days, after which the animal returns to normal.

If a larger but still sublethal, or a lethal dose is injected intravenously the animal reacts within 24 hours with subnormal temperature, considerable loss in weight, and prostration. Severe diarrhea arises, the stools being fluid and containing much mucus and more or less blood. The sensory and motor functions appear normal. The state lasts for 1 to 3 days, after which gradual recovery takes place, or death follows.

At autopsy the peritoneum is dull, its blood vessels are injected, and the peritoneal cavity contains a serous fluid. The small intestines are usually unaffected except that the vessels in the serosa may be injected. Occasionally the ileum is involved in the same extensive way as the large intestine. The walls of the latter are greatly thickened, edematous, injected, and show small discrete hemorrhages. A glairy gelatinous material covers the serous coat. On opening the intestines the contents are found to consist of blood-tinged mucus. The villi are hyperemic; the mucosa is swollen and reveals discrete hemorrhages and small ulcerations. In some instances necrotic areas are seen, and in one instance an area 2.5 cm. wide encircling the cecum was gangrenous. Microscopically destruction of the glandular elements, as well as a superficial general necrosis, is noted (Fig. 4). There is a cellular exudation in the submucosa and considerable edema and degeneration of the muscular layers. In the main, these pathologic effects in the intestine agree with the description given by Flexner and Sweet and others of the intestinal lesions produced by the injection of the whole dysentery toxin.

There are no lesions in the cerebrospinal nervous system. Hence this poison can be regarded, in contradistinction to the exotoxin, as an enterotoxin.
PETER K. OLITSKY AND I. J. KIGLER

Resistance to Heat.—A property common to endotoxins is heat stability. We have determined that Shiga endotoxin is destroyed when heated at 85-90°C. for 1 hour.

Neutralization by Antisera.—Antitoxic serum fails to neutralize endotoxin. Endotoxin, however, is neutralized by an antibacterial serum prepared by actively immunizing horses with Shiga bacilli.

To summarize, the Shiga endotoxin is a definite toxin, probably of intracellular origin, conforming to the properties of the endotoxins as a class. It differs physically and biologically from the Shiga exotoxin. Moreover, the two are separable by various procedures.

The Antitoxins of Bacillus dysenteriae Shiga.

That the exotoxin is capable of yielding an antitoxic serum is shown by the following experiment.

Experiment 8. Horse A.—Nov. 12, 1917. Injected intravenously with 5 cc. of Toxin 2 (prepared from Strain 109 grown in egg albumin broth 5 days and filtered) mixed with 1 cc. of polyvalent antidysenteric serum, as described below. Nov. 13. Injected similarly with 5 cc. of toxin but only 0.5 cc. of serum. Nov. 14. Same amount of toxin; 0.1 cc. of serum. Thereafter pure exotoxin was injected, the next series being started 7 days later with 1 cc. The intervals of injection, the increase of dosage, etc., followed the method given by Flexner and Amoss.16 Jan. 26, 1918. Dose increased to 30 cc., or a total of 80 cc. for the 3 day period of immunization. The horse reacted severely to this amount. Following this, single injections were given at weekly intervals, starting with 20 cc. and increasing slowly to 50 cc. Jan. 28. Trial bleeding; no antitoxic content. Nov., 1918. Trial bleeding; serum showed 1,000 antitoxic units per cc. Feb. 10, 1919. Trial bleeding; serum showed 2,000 antitoxic units per cc. (The basis for computation was one minimum lethal dose.)

Fig. 5 illustrates the results, selected from a series, of one of the neutralizing experiments made with this antitoxic serum. As shown by Rabbit B, the exotoxin is removed from a mixture of endotoxin and exotoxin by neutralization with the antitoxic serum; but as the endotoxin is unaffected the animal succumbed later and showed the intestinal lesions but no changes in the nervous system.

At this point a study was made of the stock polyvalent antidysenteric serum prepared at The Rockefeller Institute. This serum is ob-

tained from horses repeatedly injected with live cultures of Shiga and Flexner bacilli according to the method of Flexner and Amoss. The serum tested was obtained from horses under immunization for several years (one horse 2 years, another 5 years). It was found that although the serum was prepared by injecting the cultures, it contained at least 2,000 antiexotoxic units per cc. as well as antien-dotoxicto and other antibacterial antibodies.

SUMMARY.

With the methods which have been described we have separated an exotoxin and an endotoxin from cultures of the Shiga dysenteric bacillus. The study of the nature and effect of the poison of this microorganism is thus simplified. The two toxins are physically and biologically distinct. The exotoxin is relatively heat-labile, arises in the early period of growth, and yields an antiexotoxic immune serum. The endotoxin, on the other hand, is heat-stable, is formed in the later period of growth, and is not neutralized by the antiexotoxic serum. The exotoxin exhibits a specific affinity for the central nervous organs in the rabbit, giving rise to a characteristic lesion—mainly, hemorrhages, necroses, and possibly a perivascular infiltration in the gray matter of the upper spinal cord and medulla. The endotoxin exerts a typical action on the intestinal tract, producing edema, hemorrhages, necroses, and ulcerations, especially in the large intestine.

In dysentery in man the intestinal lesions predominate, but in severe epidemics paralysis and neuritis have been observed (Osler17).

These facts become especially significant from the standpoint of the serum therapy of bacillary dysentery. A potent antidysenteric serum should contain antibodies against the exotoxin as well as the endotoxin. That such a serum can be produced in horses has been experimentally demonstrated.

16 Four lethal doses of endotoxin were neutralized by 0.01 cc. of this serum; on the basis of one lethal unit the serum may be said to contain 400 antiendotoxicto units. The antibacterial antibodies tested were agglutinins. For some strains of Shiga bacilli the titer reached 1:20,000. In no instance was it less than 1:2,000.

EXPLANATION OF PLATES.

PLATE 6.

Fig. 1. Upper cervical region of the spinal cord of a rabbit injected intravenously with Shiga exotoxin. The hemorrhagic lesions in the gray matter, the edema, and the degeneration of the neurons are shown. × 85.

Fig. 2. Section of the medulla of a rabbit injected intravenously with Shiga exotoxin. The perivascular lesion is shown. Three capillaries, indicated by arrow-heads, are seen in different stages of round cell infiltration. × 320.

PLATE 7.

Fig. 3. Intestinal villus of a rabbit injected intravenously with Shiga exotoxin. The villus is not affected. × 72.

Fig. 4. Intestinal villus of a rabbit injected intravenously with Shiga endotoxin. The superficial necrosis of the entire villus is evident. Most of the glandular elements are destroyed and the villus is considerably atrophied. × 72.

PLATE 8.

Fig. 5. Rabbits A and B injected intravenously with filtrates containing both exotoxin and endotoxin. Rabbit A, injected with the mixtures of toxins incubated with normal horse serum, shows the effect of unneutralized exotoxin (paralysis and no intestinal symptoms). Rabbit B, injected with the mixtures of toxins incubated with antixotoxic serum, shows the effect of neutralized exotoxin and unneutralized endotoxin (no paralysis but pronounced intestinal symptoms).
(Olinsky and Klüger: *Bacillus dysenteriae* Shiga.)
(Olitsky and Kliger: *Bacillus dysenteriae Shiga.*)