# SEPARATION OF THE TOXINS OF BACILLUS DYSENTERIÆ SHIGA.

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In 1920, Olitsky and Kligler[1] separated the poison of *Bacillus dysenteriæ* Shiga into two physically and biologically distinct toxins. One, a true, soluble, exotoxin, an early product of the growth of this microorganism *in vitro*, is relatively heat-labile and yields an anti-exotoxic immune serum. The other, an endotoxin, a product of the autolysis, or disintegration, of the bacillus with the resultant liberation of intracellular components, is heat-stable and is not neutralized by antiexotoxic serum. The exotoxin has a specific affinity for the central nervous system in the rabbit; the endotoxin, on the other hand, affects only the intestinal tract.

Since the exotoxin arises in the early period of growth, and since the endotoxin results from bacterial disintegration—a factor difficult to control in growing cultures—there is great technical difficulty in preparing pure endotoxin or exotoxin directly from the Shiga bacillus. Olitsky and Kligler have indicated methods for their separation. In this paper we shall describe an additional method which we believe to be more simple and effective. In this way the previous observation that the toxins of this microorganism are distinct has been confirmed.

It is relatively more simple to obtain pure neurotoxic exotoxin. Merely the filtrate of the early growths of Shiga bacilli, before bacterial disintegration occurs, suffices. Hence young, vigorously growing, well oxygenated cultures, incubated at 37°C. for from 1 to 3 or even to 5 or 7 days, depending on the rate of autolysis, or disintegration, when filtered through Berkefeld V or N candles, yield pure exotoxin in the bacteria-free filtrate.[1] This is not the case, however, with

[1] Olitsky, P. K., and Kligler, I. J., *J. Exp. Med.*, 1920, xxxi, 19.

endotoxin. The endotoxin which arises later as a result of bacterial dissolution is always admixed with the exotoxin which is first produced in the medium, and therefore the toxins require mechanical separation for purification.

*Suppression of Exotoxin by Anaerobiosis.*

If the exotoxic function of Shiga bacilli could be suppressed, the problem of producing pure endotoxin directly from disintegrated bacilli would be simplified. In seeking for a method for this suppression, use was made of the underlying principle of rapid and increased exotoxic production—oxygenation. Roux and Yersin,[2] by passing a stream of oxygen through the cultures or employing thin layers of culture fluid, determined the requirements for the prompt and effective production of diphtheritic soluble toxin—a method subsequently yielding successful results in the hands of Park and Williams[3] and accepted generally as a standard, not only for the diphtheritic exotoxin but also for that of other bacteria, including the Shiga bacillus.[1,4] If the converse held, that is, if Shiga bacilli were grown in an oxygen-free atmosphere and in a deep layered medium, the exotoxic function would be lowered or destroyed so that mechanical disintegration of the bacterial bodies in this medium might yield pure endotoxin.

In 1904, Rosenthal[4] found that anaerobic conditions diminish the yield of Shiga bacillus poison, although no mention was made of which type of the toxin is involved. Later, Doerr[5] affirmed that under similar conditions no specific dysenteric toxin is present in filtrates. No reference was made by these investigators, however, to any toxin and its type resident in the bacterial bodies comprising the residuum.

*Experiments with Aerobic Cultures.*

Our first attempts at experimentation related to the study of the toxicity of the particular strain of *Bacillus dysenteriæ* Shiga employed,

[2] Roux, E., and Yersin, A., *Ann. Inst. Pasteur*, 1888, ii, 629; 1889, iii, 273.

[3] Park, W. H., and Williams, A. W., Pathogenic microörganisms, Philadelphia and New York, 7th edition, 1920, 342.

[4] Rosenthal, L., *Deutsch. med. Woch.*, 1904, xxx, 235.

[5] Doerr, R., Das Dysenterietoxin, Jena, 1907, 30.

Strain 109.[1] This culture was maintained in artificial media for $5\frac{1}{2}$ years, or $3\frac{1}{2}$ years after having been tested by Olitsky and Kligler.[1]

*Experiment 1.*[6]—2 per cent peptone broth was placed in a Noguchi leptospira vaccine flask—a flask made of Pyrex glass measuring 10 cm. in height, with a diameter at the neck of 1.5 cm. and at the base of 7.5 cm. The object in using this sort of vessel was to allow thorough aeration, by providing a large surface area. Sufficient medium was employed to make a layer of broth 0.5 cm. deep. The broth was adjusted to pH 7.4. We desired to avoid an alkaline reaction since it encourages autolysis, or disintegration, of the bacilli, thus liberating the intracellular endotoxin. The medium was inoculated with a loopful of *B. dysenteriæ* Shiga, Strain 109, obtained from a plain agar slant growth of 18 hours duration. The flask was incubated for 3 days at 37°C. and frequently shaken to aerate the contents. The culture was then centrifuged and the supernatant fluid decanted and filtered through Berkefeld V candles. The bacteria-free filtrate was injected into the auricular vein of rabbits weighing about 1,800 gm.

The rabbits which received 1, 2, 5, and 10 cc. died, depending on the dosage, within 5 to 24 hours, with symptoms indicative of lesions in the medulla and upper cervical cord. These lesions were noted in stained sections and consisted of extensive hemorrhages, chiefly, along with edema, necrosis, and changes in neurons already described by Olitsky and Kligler.[1] A rabbit injected with 0.4 cc. showed no symptoms until the 4th day after injection, when complete paralysis occurred in the anterior extremities with paresis of the posterior limbs, and complete paralysis of all the extremities on the 5th day. The nervous symptoms endured for 4 days; thereafter the animal returned slowly to normal. A rabbit injected with 0.35 cc. of the filtrate exhibited slight weakness of the posterior extremities 4 days after inoculation, which lasted for 1 day only. In none of all these rabbits injected with the filtrate were any intestinal symptoms noted.

At the same time, the centrifuged, sedimented bacilli were washed in sterile distilled water, recentrifuged, and the resultant deposit suspended in 5 cc. of distilled water, to which 1 per cent of sodium carbonate had been added. The mixture was then heated for 30 minutes at 56°C. and kept for 24 hours at 37°C., so as to favor autolysis. Prior to injection, the autolysate was examined by means of Gram's stain and numerous shadow forms of the bacilli were seen. Cultivation tests demonstrated that the bacilli were dead.

A rabbit injected with 0.1 cc. of the autolysate of Shiga bacilli showed 4 days after inoculation a transitory mucous diarrhea of 2 days duration from which it promptly recovered. There were no signs of nervous involvement. A rabbit inoculated with 0.5 cc. of the autolysate presented paralysis of the anterior extremities after 24 hours and died after 48 hours. Autopsy revealed the typical nervous

[6] Only typical protocols are presented of a number of repeated experiments

lesions, together with a glazed appearance of the intestines, which were edematous and congested, and the intestinal contents gave a positive benzidine test for blood. Histologically, the appearances corresponded to those already described by Flexner and Sweet,[7] Doerr,[5] Dopter,[8] and others,[1] as a result of the action of Shiga bacillus toxin.

This experiment was repeated, employing, instead of a 3 day growth of Shiga bacilli in Noguchi flasks, a 24 hour growth. The results were practically identical; the dosage of the bacteria-free filtrate required to induce nervous lesions without intestinal involvement and the dosage of the autolysate of the bacilli necessary to produce intestinal lesions combined with nervous lesions were the same as in the case of the 3 day growth.

From the above experiment it will be noted that Shiga bacilli, Strain 109, when grown in a well aerated medium, forms in 1 to 3 days a soluble toxin which is recoverable in bacteria-free filtrates. This exotoxin exerts a typical action on the central nervous organs of the rabbit, producing mainly hemorrhages and necroses in the medulla and upper cervical cord, but not affecting the intestinal tract. On the other hand, autolysates of the bacterial bodies induce not only similar nervous lesions but also intestinal involvement. Therefore exotoxin, or neurotoxin, a product of the growth of Shiga bacilli, can be obtained in a pure state by this method; but endotoxin, or enterotoxin, the result of bacterial disintegration, is always admixed with a certain amount of exotoxin.

### *Experiments with Anaerobic Cultures.*

The next experiments concerned the complete suppression of the neurotoxin-producing activity of Shiga bacilli by employing anaerobic methods and a study of the disintegration products of the bacterial bodies in such cultures.

*Experiment 2.*—For producing anaerobic conditions either of two methods, both giving practically the same results, was employed. The first method consisted in the inoculation of a tall column (15 cm.) of 1 per cent dextrose broth, pH 7.4, in a Noguchi test-tube, with a loopful of Shiga bacilli, Strain 109, grown pre-

[7] Flexner, S., and Sweet, J. E., *J. Exp. Med.*, 1906, viii, 514.

[8] Dopter, M. C., Les dysenteries, Paris, 1909, 75 ff.

viously for 18 hours in Brown's anaerobic jar.[9] The inoculated tubes were then placed in a Brown jar and incubated for 24 hours at 37°C. The growth of this tube was then subplanted into another and kept under similar anaerobic conditions. In this way the Shiga bacilli were subplanted to four consecutive anaerobic cultures, each incubating for 24 hours at 37°C. The fourth subplant was employed in the following experiment. The other method for obtaining anaerobic conditions consisted of layering over a similar column of the broth with petrolatum, then placing in an Arnold sterilizer and heating for ½ hour at 100°C. The medium was now anaerobic and was inoculated through the seal with 0.1 cc. of a Shiga bacillus broth culture grown previously for 18 hours in a Brown anaerobic jar. The inoculated, sealed tubes were kept for 24 hours at 37°C. With these exceptions, the method corresponded exactly with the one in which the Brown jar replaced the petrolatum seal for maintaining anaerobic conditions.

These anaerobic cultures were centrifuged at high speed until the supernatant fluid was clear. The latter was decanted, filtered through Berkefeld V or N candles, and the bacteria-free filtrate inoculated into the auricular vein of rabbits.

Rabbits which received 0.5, 1, 2, and 4 cc. of this anaerobic bacteria-free filtrate were unaffected and remained free from nervous or intestinal symptoms for a period of observation lasting 2 weeks or more.

The centrifuged, sedimented bacilli of these anaerobic cultures were, at the same time, washed in sterile distilled water, recentrifuged, and the clear supernatant fluid, or washings, decanted. The solid sediment of bacilli was suspended in 5 cc. of distilled water to which 1 per cent of sodium carbonate had been added. The mixture was heated for 30 minutes at 56°C. so as to kill the microorganisms, and then kept for 24 hours at 37°C. to favor autolysis. Prior to injection the autolysate was stained by Gram's method and cultured, and the mixture was found to consist of either completely autolyzed or dead bacilli. This material, in doses of 0.1, 0.2, 0.25, and 0.5 cc., was injected into the auricular vein of rabbits. The animals showed pronounced intestinal symptoms from 24 to 48 hours after inoculation, depending on the dosage. The first sign to be noted was straining and tenesmus, which was continual, accompanied by frequent evacuations of small, semisolid masses of feces. As the condition progressed, there was a mucous diarrhea streaked with either visible or microscopic blood, determined by the benzidine test. This condition persisted for 2 to 5 days, the animals returning, thereafter, to normal. Indeed, none of this series died. Some of the animals were killed at the height of the reaction for macroscopic and histopathological examination. The intestines then revealed grossly the typical glazing of the peritoneal coat, the edema, the injection of blood vessels, and hemorrhages, and showed microscopically the edema, hemorrhages, necroses, ulceration, and fibrinous (diphtheritic) covering of the epithelial coat, corresponding to the description given by Flexner and Sweet,[7] Doerr,[5]

[9] Brown, J. H., *J. Exp. Med.*, 1921, xxxiii, 677; 1922, xxxv, 467.

and Dopter[8] of the action of the Shiga poison, and later by Olitsky and Kligler[1] of the effects of Shiga endotoxin. In none of these animals were noted any signs of nervous involvement, either clinically or in section, even when ten times (1 cc.) of the minimal enterotoxic dose was used.

This experiment, typical of four similar ones, demonstrates that the exotoxic function of Shiga bacilli can be suppressed by growing the microorganisms under anaerobic conditions, either in fluid medium under a petrolatum seal or in this medium placed in an anaerobic jar. The bacteria, now in an exotoxin-free environment, can be autolyzed, or dissolved, with the resultant liberation of intracellular components, or endotoxin. The endotoxin, the preparation of which does not differ essentially from that of endotoxins from other bacteria, has a specific affinity for the intestinal tract, producing edema, hemorrhages, necroses, and ulcerations. The central nervous organs are not affected; hence the endotoxin can be properly designated as an enterotoxin.

We have thus separated the two toxins of the strain of *Bacillus dysenteriæ* Shiga which has been employed and have shown their biological distinctions. We now proposed to extend the observations of Olitsky and Kligler[1] by studying additional physical differences.

### *Diffusion of Toxins through Permeable Membranes.*

The diffusion of the toxins through the walls of collodion sacs was investigated.

*Methods.*—Permeable collodion sacs for intraabdominal implantation and those for bacterial cultivation *in vitro* were prepared, following closely Gates' method.[10,11] For intraperitoneal implantation, the sacs were made on No. 12 size veterinary capsules;[10] for cultivation *in vitro*, the sacs were prepared in flasks.[12]

Preliminary experiments resulted in the choice of 2 per cent peptone broth (pH 7.4, for the reasons stated above) instead of the egg albumin broth which was suggested by Olitsky and Kligler[1] as the most effective medium. While the contents of the sac containing cultures of Shiga bacilli in the albumin broth were highly

[10] Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 25.

[11] Gates, F. L., *J. Exp. Med.*, 1922, xxxv, 635.

[12] Gates,[11] p. 640.

toxic for rabbits, dialysates contained less toxin than that obtained by the use of a clear simple medium such as the peptone broth. The dense cultures of the bacilli in albumin broth coat the inner surface of the sac, thus preventing active dialysis.

*Experiments with Sacs in Vivo.*—Five rabbits were employed and into the abdominal cavity of each were placed two sealed sacs containing the broth inoculated with two loopfuls of agar slant cultures of Shiga bacilli. Four of the rabbits were unaffected for a period of 1 month. Then the sacs of two of the animals were removed and the contents were examined. A growth of Shiga bacilli was obtained on transplanting to agar, although a number were found autolyzed in stained preparations of the material. The contents of the sacs from one rabbit were reinjected into the auricular vein of two normal animals in doses of 0.1 and 0.05 cc. These latter died in 48 hours with typical nervous and intestinal symptoms. One of the rabbits containing the sacs was also injected at the same time with 0.1 cc. of the sac contents. This animal was unaffected. The experiment was repeated with similar results.

One of the four rabbits unaffected by harboring the sac cultures showed, 1 month after implantation, an agglutination serum titer of 1:1,280 against Shiga bacilli. The remaining three were negative.

The fifth rabbit exhibited, 5 days after implantation of the sacs, paralysis of both anterior extremities, paresis of the posterior extremities, and hyperesthesia. There were no intestinal symptoms. The animal died the next day, showing complete paralysis but no intestinal symptoms. The histopathology revealed the typical nervous lesions but the intestinal tract was normal. That these effects were not due to breakage of the sac and liberation of the bacteria was proved by culturing the sealed sacs after removal from the body for 48 hours in dextrose broth. No growth of Shiga bacilli occurred in the surrounding medium.

From these experiments it may be concluded that the Shiga bacilli, when grown in fluid medium contained in a sac which is implanted intraabdominally, survive and multiply therein for at least 1 month. During this period different effects are produced, depending, we believe, on the state of permeability of the sac. Rabbits harboring highly permeable sacs show the results of dialysis of pure exotoxin within 5 days after implantation; that is, the animal succumbs to lesions produced in the medulla and upper cervical cord. One may assume that if the exotoxin would dialyze out of the sac in nonlethal amounts the rabbit might exhibit at a later time intestinal symptoms due to subsequent disintegration of the bacilli within the sac with formation of endotoxin. Rabbits containing sacs of a lesser degree of permeability, although sufficient to allow an active diffusion

of the body fluids through their walls, produce a high titer of agglutinins in their blood sera. In this respect we have been able to confirm a previous observation of Gates,[13] who found that typhoid bacilli survive and multiply in sacs *in abdomine* for $1\frac{1}{2}$ years; during this time agglutinins for these bacilli are produced. Even when agglutinins are not detectable in the serum of these animals, they remain immune to a later injection of either exotoxin or endotoxin.

The difficulty of determining the fine degrees of permeability of the sacs necessary to induce these effects renders this method unsatisfactory for our purposes of separating the two dysenteric toxins. However, certain conclusions may be drawn from these experiments: The contents of the sacs after 1 month *in abdomine* are highly toxic for normal rabbits, which show the effects of both exotoxin and endotoxin, corresponding to the multiplication and autolysis and dissolution proceeding in the sacs. Exotoxin diffuses through, primarily causing the death of the animal by the typical nervous lesions. Agglutinins are produced in surviving animals, in one instance in a titer of 1:1,280. Finally, immunity is conferred in the latter to subsequent injections of exotoxin and endotoxin.

*Experiment with Sacs in Vitro.*—For the purposes of these experiments, Gates' method of preparing sacs in flasks[12] was employed. 30 cc. of the same medium employed in the intraabdominal sacs were placed within the sac and 30 cc. of distilled water without. The medium within the collodion membrane was inoculated with two loopfuls of Shiga bacilli, Strain 109, grown for 18 to 24 hours on agar slants. The cultures were then kept for varying periods of time (from 3 to 8 days) at 37°C.

The cultures which were incubated 3, 4, and 5 days produced in the dialysate the typical exotoxin which induced in normal rabbits neurotoxic symptoms but no intestinal involvement. A single protocol is presented to show the manner in which an experiment was conducted and the results obtained. The protocol relates to one of a series of experiments made with a 5 day culture. The cultures kept at 37°C. for 3 and 4 days gave practically the same results, except that the nervous symptoms were of a milder degree and more transitory, enduring for 2 to 3 days, the animal returning thereafter to normal.

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[13] Gates, F. L., personal communication.

*Experiment 3. 5 Day Culture.—*

| Rabbit. | Material injected intravenously. | Dose. | Symptoms and course following injection. |
|---|---|---|---|
| | | *cc.* | |
| A | Dialysate. | 10 | 48 hrs., paresis of right anterior extremity. 72 hrs., paralysis of both anterior extremities, hyperesthesia; paresis of posterior extremities. 4 days, condition unchanged. 5 days, condition improved; partial paralysis of anterior extremities; paresis of posterior extremities. 10 days, practically recovered. No intestinal symptoms during the 10 days. |
| B | " | 10 | 48 hrs., hyperesthesia, paresis of posterior extremities. 4 days, paresis of anterior and posterior extremities. 7 days, partial paralysis of right anterior extremity; other limbs recovered. 11 days, practically recovered. No intestinal symptoms during the 11 days. |
| C | " + polyvalent antidysenteric serum* (injected simultaneously). | 10<br>5 | No effect. |
| D | Dialysate + polyvalent antidysenteric serum* (injected simultaneously). | 10<br>5 | " " |
| E | Dialysate heated at 75°C. for 1 hr. | 10 | " " |
| F† (immune). | Dialysate injected into rabbit which recovered from exotoxic paralysis 3 wks. previously. | 10 | " " |

| Rabbit. | Material injected intravenously. | Dose. | Symptoms and course following injection. |
|---|---|---|---|
| | | *cc.* | |
| G | Contents of sac.‡ | 2 | 24 hrs., paresis of right anterior extremity; tenesmus. 48 hrs., paralysis of anterior extremities; paresis of posterior extremities; mucous diarrhea. 72 hrs., complete paralysis; diarrhea. Moribund; chloroformed. Nervous and intestinal lesions typical. |

* This serum was the only therapeutic serum available. It was the Rockefeller Institute serum and contained exotoxic, endotoxic, and bacterial antibodies.[1]

† In another series of similar experiments with 5 day cultures, four animals which had been injected 2 to 3 weeks previously with exotoxin and had recovered from its action were reinjected with dialysate. They were all unaffected.

‡ On microscopic examination, the sac contents showed evidence of bacillary disintegration.

These experiments, which were repeated with similar results, demonstrate that the exotoxin is the first to appear in the bacteria-free dialysate. The exotoxin was identified by its power to induce nervous lesions, by its relative thermolability,[1] and by its failure to act in animals previously recovered from exotoxic effects (immunity tests), or when the toxin was injected simultaneously with immune serum. In the animals injected with the dialysates, no intestinal signs were observed, although on the 5th day the contents of the sac showed beginning autolysis, or disintegration, of the Shiga bacilli, and, consequently, caused both nervous and intestinal symptoms when injected into rabbits. However, after this period, and corresponding with an increasing degree of dissolution of the bacilli within the collodion membrane, both exotoxin and endotoxin appeared in the dialysates, as the following protocol shows.

*Experiment 4. 6 to 8 Day Cultures.*—Cultures such as were employed in Experiment 3 were allowed to incubate for 6, 7, and 8 days at 37°C. Normal rabbits were then injected with the different components of the cultures, as represented in the following protocol illustrating an experiment with a 6 day culture.

| Rabbit. | Material injected intravenously. | Dose. | Symptoms and course following injection. |
|---|---|---|---|
| | | *cc.* | |
| A | Dialysate. | 10 | 24 hrs., paresis; mucous diarrhea. 48 hrs., paralysis and diarrhea. 72 hrs., completely paralyzed. Killed. Lesions in nervous and intestinal organs typical. |
| B | " | 5 | 24 hrs., paresis of left anterior extremity and posterior extremity. 48 hrs., condition unchanged for 7 days thereafter. During the 9 days, no intestinal symptoms observed. |
| C | " heated for 1 hr. at 75°C. | 10 | Showed no nervous symptoms for 9 days; mucous diarrhea 3 days after injection which lasted 3 days; animal recovered thereafter. |
| D | Sac contents (containing numerous shadow forms along with viable Shiga bacilli). | 1 | 24 hrs., paresis; mucous diarrhea. 48 hrs., paralysis of anterior extremities; paresis of posterior extremities; tenesmus; mucous diarrhea. Moribund; killed. Lesions in medulla, upper cervical cord, and intestines typical. |

Experiments with 7 and 8 day cultures gave similar results, except that it required only 5 cc. of the dialysate to induce intestinal symptoms.

It appears from Experiment 4, therefore, that *in vitro* cultures of Shiga bacilli in collodion sacs produce mixtures of exotoxin and endotoxin in the bacteria-free dialysates. Hence, the early growths, that is up to 5 days incubation, yield pure exotoxin (Experiment 3) which diffuses readily through the collodion membrane and is detectable in the dialysate. After this period not only exotoxin but endotoxin diffuses through and the rate of the latter is proportional to the amount of dissolution, or disintegration, of the Shiga bacilli within the membrane. From the foregoing experiment it will be noted, as well, that the thermostable endotoxin in the dialysate can be separated from the relatively thermolabile exotoxin by heat.[1]

## DISCUSSION.

Additional methods are presented to support the observations of Olitsky and Kligler[1] concerning the dual nature of the poison of Shiga bacilli. The first method consists of the suppression of the exotoxic, or neurotoxic, function of the microorganisms by anaerobiosis so that the disintegration of the bacteria, with consequent liberation of intracellular components, yields pure endotoxin, or enterotoxin. Thus the difficulty of securing pure endotoxin directly from the culture medium is overcome, for hitherto this toxin was always found admixed in the medium with exotoxin, which is an early product of the growth of Shiga bacilli.

The second method relates to the diffusion of these toxins through the walls of collodion sacs implanted intraabdominally or placed in flasks. Cultures in sacs implanted intraabdominally show different effects, depending on the permeability of the collodion membrane; highly permeable sacs allow the early passage of exotoxin, somewhat denser membranes permit only the diffusion of substances inducing agglutinins or immunity. Cultures in sacs placed in flasks yield in the fluid surrounding the membrane first exotoxin and later endotoxin. The passage through the membrane of endotoxin depends on the rate of bacterial disintegration within the sac.

Furthermore, the biological and physical differences of the two toxins which have already been demonstrated[1] are confirmed.

A practical bearing of this work concerns the preparation of an effective Shiga antidysenteric serum. The experimental production of antiexotoxin has already been described.[1] A potent serum for general use, however, should be anti-infectious and should contain endotoxic and exotoxic antibodies. The injection intravenously in horses of live Shiga bacilli, after the manner described by Flexner and Amoss,[14] has yielded a serum which contains 2,000 antiexotoxic units per cc., as well as endotoxic and bacterial antibodies (0.01 cc. of the serum neutralizes four minimal endotoxic doses and shows agglutinins ranging from 1:2,000 to 1:20,000 against Shiga bacilli[1]). Its anti-infectious potency as measured by Flexner and Amoss is such that 0.008 cc. protects rabbits against two lethal doses of Shiga bacilli.

[14] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1915, xxi, 515.

## CONCLUSIONS.

By the suppression, through anaerobiosis, of the exotoxin-producing activity of *Bacillus dysenteriæ* Shiga a pure endotoxin is produced directly from the culture. The duality of the poison of Shiga bacillus is further substantiated by studies on the diffusion of exotoxin, or neurotoxin, and endotoxin, or enterotoxin, by means of collodion sacs, implanted intraabdominally in rabbits or placed *in vitro*.