STUDIES ON THE TOXIN PRODUCTION OF THE SHIGA BACILLI

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PLATES 1 AND 2

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In 1903 Vaillard and Dopter (1) demonstrated that the Shiga bacillus is extremely toxic for rabbits. Sterile filtrates of 14 day old broth cultures injected intravenously into rabbits, gave rise to diarrhea, paralysis of the extremities, and death. At autopsy they found congestion of the intestine, mucus, and blood adhering to the mucosa. The walls of the gut, especially of the cecum, were thickened and edematous, and frequently they found small hemorrhages and ulcerations in the mucosa. Dopter (2), in 1905, studying the histological appearances of the animals dying of paralysis, observed distinct lesions of the spinal cord, consisting of chromatolysis and sometimes focal necroses of the grey matter of the anterior horn.

The toxin of the Shiga bacillus thus has, according to the clinical and anatomical examinations of Vaillard and Dopter, a special effect upon the nervous system and the intestinal tract. The Shiga toxin has been studied by a number of bacteriologists, notably by Conradi (3), Shiga and Neisser (4), Todd (5), Kruse (6), Kolle, Heller, and de Mestrale (7), and Selter (8). It was shown that this toxin is highly active in rabbits and horses, but has less effect on guinea pigs, rats, and mice. Some of the authors found that the toxin in rabbits mainly affected the intestinal tract, while others described the paralysis of the limbs previously noted by Vaillard and Dopter.

There was, however, a disagreement between the workers as to the precise nature of the toxin. This was possibly due to the fact that the different authors used different methods in preparing their toxins. Some of them regarded it as an endotoxin, and they prepared it by washing off the growth on agar slants in saline solution, heating to 56° or 60°C., incubating for 24 to 48 hours, and filtering through Berkefeld candles. Others regarded it as an exotoxin, and they obtained it by growing the bacilli in alkaline broth for a period of 2 to 6 weeks, and then filtering. The various workers obtained, however, the same effect on rabbits with the autolyzed bacilli and the filtrates of broth cultures. It was maintained by Klein (9) that it was possible to produce antitoxins against both kinds of toxin.

In 1920, Olitsky and Kligler (10) described methods with which they main-

tained that they were able to separate an exotoxin and an endotoxin from the Shiga bacillus. The exotoxin was derived from 4 to 7 day old broth cultures filtered through Berkefeld candles. It was heat labile, and produced lesions of the central nervous system in rabbits, without at the same time injuring the intestines. The period of incubation of the broth cultures had an important bearing on the nature of the toxic product, as an incubation for 14 days to 3 weeks leads to autolysis of the bacteria and formation of endotoxin. The pure endotoxin exerted a typical action on the intestinal tract, producing edema, hemorrhages, and ulcerations, particularly in the large intestine. It was, however, difficult to prepare a pure endotoxin. When the bacterial growth on agar was washed off in saline solution, and the killed bacterial bodies were autolyzed for 2 days at 37°C., and filtered through Berkefeld candles, usually both toxins were present in the filtrate. The removal of the exotoxin could be carried out by heating the toxic autolysate at 80°C. for 1 hour. The endotoxin was more heat resistant than the exotoxin. Furthermore they found that the two toxins produced different antitoxins. According to the experiments of Olitsky and Kligler, the exotoxin behaved as a pure neurotoxin, and the endotoxin as a pure enterotoxin. Previous workers had not succeeded in demonstrating the distinct biological difference between the two toxins, and Olitsky and Kligler believed this to be due to the methods usually employed in preparing the toxins. This seemed especially likely in the case of the exotoxin, as the previous authors usually had incubated the broth cultures 2 to 3 weeks.

More recent workers, who have studied the poisons of the Shiga bacillus, have paid little attention to the investigations of Olitsky and Kligler. This is particularly the case with those authors who have studied the toxin production of the "S" and "R" forms. Dudgeon and Hope Simpson (11), as also Sudmerson, Rung, and O'Brien (12), found that the S and R variants of the Shiga bacillus were equally toxic. These authors did not, however, prepare endotoxin and exotoxin according to the methods described by Olitsky and Kligler. In the case of other intestinal rods, on the other hand, the conditions seem to be the opposite, as, according to Ibrahim and Schütze (13), the S forms usually are more toxic than the R variants.

In a detailed study on the dissociation of the dysentery bacilli, we (14) described several variants. The different variants could be grouped in three serological types and designated S, R, and R_n. The S forms, which were agglutinated in large clumps in homologous immune serum, and the fine agglutinating R forms, were types previously well known from the publications of Arkwright (15) and others. The R_n forms, on the other hand, have not been observed, as yet, by other workers. They lacked the ability to produce antibodies after inoculation into rabbits, and, from this type, we have not succeeded in pre-

paring useful antigens for complement fixation and agglutination tests. The R_n colonies resembled in some respects the G forms of Hadley and his coworkers (16), but they were not, like the G type, filtrable. The S and R forms of the Shiga bacillus, which differed completely from each other, gave no agglutination or complement fixation with the serum of the R_n variant. Hence we have found it interesting to test the toxicity of these serologically different variants. We have tested both the endotoxin and the exotoxin, following the technic of Olitsky and Kligler, as, *a priori*, one might suppose that any possible difference in toxicity then might appear. Especially we were inclined to believe that the endotoxin might depend upon the antigenic behavior of the variants. The results we obtained deviated so far from those of the American authors, that we have found it necessary to undertake detailed anatomical examinations. In the present paper we will deal with these toxin experiments and the consequent histological studies.

Material and Methods

We have used three different strains of the Shiga bacillus. Two of them were isolated in Norway (Strain Røken, 1921 and Strain Aas, 1931), and one strain was obtained from the Lister Institute in 1920. The origin of this strain is unknown. All had the typical cultural characteristics. There was little or no dissimilarity between the toxins of the different strains, and most of the experiments were therefore carried out with the Aas strain. The cultures were of course not those employed by Olitsky and Kligler.

Before preparing the toxins, the S, R, and R_n strains to be tested were seeded on agar plates. Single colonies were fished in four to five consecutive generations, to ascertain that the variants were pure. Furthermore, the morphological, serological, and other properties were tested before preparing the toxins. After this control of the variants, they were subcultivated on agar slants.

Preparation of Endotoxin.—Agar cultures of the variants, controlled as described above, were seeded on agar plates, diameter 9 cm., and cultivated for 24 hours. The growth was washed off with 7 cc. normal saline for each agar plate. The bacterial emulsions in different tests were as nearly as possible made up to the same density. The bacteria were killed by heating in the water bath for $\frac{1}{2}$ hour at 60°C., and the emulsions of killed bacteria were incubated at 37°C. for 2 days, and then filtered through a Berkefeld candle. The clear yellowish filtrate was tested for sterility, and was never kept more than 1 to 2 days before use.

Preparation of Exotoxin.—The variants were cultivated in 50 cc. broth in large bottles, with a diameter of 10 cm. at the bottom. The bottles were kept in the incubator for 3 to 7 days. Good aeration, according to Olitsky and Kligler,

should favor the production of toxin. The cultures were filtered through Berkefeld candles, tested for sterility, and used the following day.

As culture media we have used agar and broth free of sugar, with a reaction set up at pH 7.4 (in the production meat was used (never Liebig extract) and to this 0.2 per cent sodium phosphate and 1 per cent Parke, Davis peptone was added).

The toxins were tested on rabbits and mice. The rabbits were injected intravenously, and the mice intraperitoneally. In all 300 rabbits and about 150 mice were used. The animals were observed several times every day. Autopsy was carried out as soon as possible after death, and specimens from several organs were removed for histological examinations (brain, spinal cord, heart, large and small intestines, liver, spleen, and kidneys).

Dose	S	R	S	R	S	R	R _n	R _n
0.0025 G.					□	□	□	□
0.004 "			□	□	□	□	■	■
0.008 "	□	□	□	□	□	□	■	■
0.016 "	■	■	□	□	□	□	■	■
0.032 "	■	⊗	■	⊗	■	⊗		
0.064 "	■	■	⊗	■	■	■	■	■
0.128 "	■	■	■	■	■	■		
0.25 "	■	■	■	■	■	■	■	■
0.5 "	■	■	■	■	■	■		
1 "	■	■	■	■	■	■	■	■

In the charts each column represents one series of animals tested, one animal per dose. The columns which represent parallel tests are separated from the others by thick lines.

■ Dead within 5 days.

⊗ Sick for some days, but recovered.

□ No symptoms.

CHART 1. Endotoxin experiments in rabbits.

Great care was taken to use animals of the same weight and of the same breed. In comparing the toxicity of the different variants, it was desirable to use numerous series of animals for each toxin. It was, however, impossible to obtain many rabbits of the same weight and of the same breed at the same time. For this reason we could do no more than test two or three series only on the same day (S, R, and R_n, one series for each).

Comparison of the Toxicity of the Different Variants

Endotoxin.—Chart 1 demonstrates different series in parallel of endotoxin experiments. The first two series (S and R) are parallel and comparable because the emulsions used in preparing the toxins had the same density, and the animals were chosen according to the requirements mentioned above. Those tests in

the charts which are parallel, are separated from the others by heavy lines. In the first two series, the minimal lethal dose is 0.016 cc., both in the S and the R toxin. In the next tests, the dose of 0.032 cc. killed the animal in the case of the S toxin, while the corresponding animal in the R toxin experiment was sick for some days. In the third set of two groups, the minimal lethal dose of the S toxin was 0.016 cc. This dose did not affect the rabbit in the case of the R toxin. 0.032 cc. R toxin caused transient symptoms, the minimal lethal dose being 0.063 cc.

The last two series in Chart 1 represent tests carried out with two R_n variants. The minimal lethal dose was in both cases 0.004 cc., and the R_n type might therefore be considered as being more toxic than the others. However, this difference cannot be stressed. The R_n tests are not parallel with the others, as neither the emulsions nor the animals were compared beforehand. In the work of Chart 2, on the other hand, parallel tests with S, R, and R_n endotoxins in mice were carried out. The S toxin seems to be the most toxic, the R_n toxin is slightly less effective,

Dose	S	R	R_n	S	R	R_n	S	R	S	R
0.004 cc.	□	□	□	□	□	□	□	□	□	□
0.008 "	□	□	□	□	□	□	□	■	□	■
0.016 "	□	□	□	□	□	□	■	■	■	■
0.032 "	■	□	□	■	□	□	■	■	■	■
0.063 "	■	□	■	■	□	■	■	■	■	■
0.125 "	■	■	■	■	■	■	■	■	■	■
0.25 "	■	■	■	■	■	■	■	■	■	■
0.5 "	■	■	■	■	■	■	■	■	■	■
1 "	■	■	■	■	■	■	■	■	■	■

CHART 2. Endotoxin experiments in mice.

while the R is weaker. In the last experiments reported in Chart 2, however, the R toxin proved more efficient than the S. The minimal lethal dose of the R was in both tests 0.008 cc., and in the S 0.016 cc.

There is evidently reason to consider the endotoxins of the three variants S, R, and R_n as about equally toxic. The differences which appeared were small, and due probably to the varying resistance of the animals.

Exotoxin.—Chart 3 gives the results of the exotoxin experiments performed with the S and R variants. In the first two parallel series, the broth cultures had been incubated for 3 days, and the minimal lethal dose of the S toxin was 0.5 cc. The same amount of R toxin caused transient symptoms, while the rabbit receiving 0.25 cc. died. In the remaining parallel tests with cultures 4 to 6 days old, there was no difference in the minimal lethal dose between the S and R exotoxin.

When the experiments in mice are taken into consideration, on the other hand it might be objected that the R exotoxin is more effective than the S. This conclusion is, however, not correct. The mice are extremely variable in their resistance to the toxins of the Shiga bacillus, as Chart 4 shows. In these tests the minimal lethal dose varies from 0.25 cc. to 0.016 cc. with the same toxin.

Dose	Rabbits										Mice			
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
0.004 cc.														
0.008 "														
0.016 "														
0.032 "														
0.063 "														
0.125 "														
0.25 "														
0.5 "														
1 "														
Age of the filtrates	3 days		4 days		6 days		6 days		6 days		6 days		6 days	

CHART 3. Exotoxin experiments in rabbits and mice.

Dose	Series		
	1	2	3
0.004 cc.			
0.008 "			
0.016 "			
0.032 "			
0.063 "			
0.125 "			
0.25 "			
0.5 "			
1 "			
Age of the filtrates	6 days		

CHART 4. Exotoxin experiments in mice. Three parallel series with one and the same "S" toxin.

These results give ground for the view that the S and the R exotoxins are equally toxic.

We have not found it necessary to carry out serial experiments with R_n exotoxin. A few rabbits only were injected, and the minimal lethal dose seemed to vary from 0.125 cc. to 0.032 cc.

Effect of Endotoxin and Exotoxin upon Rabbits

In testing the toxicity of the different variants we observed symptoms and anatomical changes in the rabbits which were entirely dissimilar to those described by Olitsky and Kligler. For example, we were unable to produce endotoxins which affected the intestinal tract only. The endotoxins which were not heated to 80°C. for 1 hour, injured the intestinal tract and several other organs including the nervous system. Usually the lesions of the nervous system dominated in the clinical picture and postmortem examinations. The unheated endotoxins never failed to affect the spinal cord (Table I).

TABLE I
"S" Endotoxin in Rabbits. Toxin Not Heated

Injected intravenously	Weight	Effect	Histological changes in
cc.	gm.		
0.25	1500	Died within 20 hrs.	Intestinal tract, spinal cord, kidneys, and liver
0.125	1725	Died within 30 hrs. Paralysis and diarrhea	Intestinal tract, spinal cord, less in kidneys and liver
0.063	1475	Died within 20 hrs.	" "
0.032	1350	Diarrhea, loss in weight, recovered	" "
0.016	1400	Died after 48 hrs. Paralysis and diarrhea	Intestinal tract, spinal cord. Kidneys and liver not examined
0.008	1325	" "	" "
0.004	1200	Died within 48 hrs. Paralysis	" "
0.002	1150	Died after 48 hrs. Paralysis	" "

By heating the endotoxins to 80°C. for 1 hour, Olitsky and Kligler succeeded in removing this injurious effect upon the nervous system, though the toxins still were able to affect the intestinal tract. We do not agree with the American authors in this respect. In our hands the heating to 80°C. for 1 hour only attenuated the poisonous effect of the endotoxin (Table II). After heating the endotoxin, the dose of 1 cc. was fatal, while 0.002 cc. caused death before heating. The doses of 0.5, 0.25, and 0.125 cc. caused transient symptoms, while the rest of the rabbits were unaffected. Furthermore as Table II shows, the injurious effect upon both the intestinal tract and the nervous

system is weakened by heating the endotoxin. After injection of 1 cc. of this heated toxin, the rabbit shows both diarrhea and paralysis of the extremities, and at autopsy we succeeded in demonstrating the typical changes in the spinal cord.

In the experiments summarized in Tables I and II, we are dealing with a very powerful endotoxin, as, before heating, the dose of 0.002 cc. was fatal. Usually our endotoxins were not so powerful (Chart 1). When these less poisonous endotoxins were heated to 80°C. for 1 hour, 5 to 10 cc. could be injected intravenously into rabbits, without causing definite symptoms. The rabbits only lost 100 to 150 gm. in

TABLE II
*"S" Endotoxin in Rabbits. The Same Toxin as in Table I, Heated at 80°C.
 for 1 Hour*

Injected intravenously	Weight	Effect	Histological changes in
cc.	gm.		
1	1700	Died after 24 hrs. Diarrhea and paralysis	Intestinal tract, spinal cord, kidneys, and liver
0.5	1600	Loss of 300 gm. in weight. No distinct symptoms	
0.25	1700	Loss of 300 gm. in weight. Diarrhea, recovered	
0.125	1675	Loss of 100 gm. in weight. No distinct symptoms	
0.063	1375	No symptoms	
0.032	1300	" "	
0.016	1300	" "	

weight. It follows that the rabbits reacted to this inoculation of the heated toxins very well.

Our results in exotoxin experiments do not agree either with those of Olitsky and Kligler. Thus the filtrates of 3, 4, and 6 day old broth cultures affected the central nervous system as well as the intestinal tract (Table III). When the animals survived more than 20 hours, both diarrhea and paralysis of the extremities were observed. The changes in the intestinal tract were as important in the case of the exotoxin as in the endotoxin experiments. On the whole there was no difference between the two toxins. The exotoxin especially always

showed some effect upon the intestinal tract. This was also the case with the filtrates of the young cultures (3 to 4 days).

Turning to the neutralization experiments, the antitoxins produced by inoculating heated endotoxin into rabbits, protected rabbits fairly well against the exotoxin. Table IV demonstrates that S and R anti-endotoxin gave the same protection against S exotoxin. Conversely, the effect of the R exotoxin was distinctly reduced both after neutralization with S and with R anti-endotoxin.

These antitoxins, produced against the heated toxins, were, however,

TABLE III
"S" Exotoxin in Rabbits

Injected intravenously	Weight	Effect	Histological changes in
cc.	gm.		
4	1800	Died within 20 hrs.	Intestinal tract, spinal cord, kidneys, less in the liver
1	1625	Died after 48 hrs. Diarrhea and paralysis	Intestinal tract, kidneys, liver. Spinal cord not examined
0.125	1650	Died after 60 hrs. Diarrhea and paralysis	Intestinal tract, spinal cord, kidneys, less in the liver
0.063	1600	Died after 72 hrs. Paralysis	" "
0.032	1400	Loss of 200 gm. in weight. Paralysis, recovered	
0.016	1500	Loss of 100 gm. in weight. Paralysis, recovered	
0.008	1250	No symptoms	

The broth culture was incubated 6 days and then filtered through Berkefeld candle.

not always very powerful, but their efficiency was increased when the inoculation of heated toxins was followed by injections of unheated toxins. As previously mentioned, the animals reacted to the immunization with the heated toxins much better than with the unheated ones. Immunization with unheated toxins or vaccines of the Shiga bacillus will often cause considerable loss in weight or be fatal. When the animals are treated with heated toxins beforehand, they are resistant to prolonged inoculation with toxins which are not detoxicated. The heating of the toxins of the Shiga bacilli has therefore proved to be useful in the production of antibacterial and antitoxic sera.

Anatomical Changes Caused by the Toxins

Organs were removed for histological examination, in order to study the microscopical lesions in the rabbits. The most important injuries were found in the spinal cord and intestinal tract, a fact in correspondence with the clinical picture. We were able, however, to demonstrate lesions in other organs, notably the heart, liver, and kidneys. The spleen and suprarenal glands, on the other hand, were histologically normal.

TABLE IV
Neutralization Experiments with Exotoxin and Anti-Endotoxin

Exotoxin	Anti-endotoxin	Effect
cc.	cc.	
0.2 S	0	Died after 24 hrs.
0.2 S	0.1 S	No symptoms
0.2 S	0.01 S	Transient paresis
0.2 S	0.1 R	No symptoms
0.2 S	0.01 R	Transient paresis
0.2 R	0	Died within 24 hrs.
0.2 R	0.1 S	No symptoms
0.2 R	0.01 S	Died within 24 hrs.
0.2 R	0.1 R	No symptoms
0.2 R	0.01 R	No symptoms

The antitoxin was serum derived from a rabbit injected intravenously with endotoxin heated to 80°C. for 1 hour.

The antitoxins were permitted to act upon the toxins for $\frac{3}{4}$ hour before injecting the rabbits.

0.2 cc. S and R toxin represented 4 to 5 minimal lethal doses.

Nervous System.—Sections from different parts of the brain showed a slight hyperemia, but never hemorrhages. For the rest, the white and gray matter of the brain was intact.

In the spinal cord, including the medulla oblongata, we observed grave lesions. The gray matter, especially in the anterior horns and the motor nuclei of medulla oblongata, was affected to a varying degree. The neurons were partly swollen, with large Nissl bodies (Fig. 1). Sometimes the cells were small, sclerotic, and homogeneously stained (Fig. 2). In the more severe cases, degeneration and necrosis appeared, with result that a few degenerated cells only were visible (Fig. 3). Hyperemia was not usually observed, and hemorrhages only once.

These lesions could be demonstrated in all parts of the spinal cord, but to a varying degree in the different regions.

Olitsky and Kligler have pointed out, however, that hemorrhages in the nervous tissue are regularly present after injection of exotoxin.

Intestinal Tract.—In the small intestines necrosis of the superficial cells was noted. A varying number of leucocytes invaded the mucosa, and the glandular elements were destroyed. Usually the walls of the gut were somewhat congested (Fig. 4).

The large intestines, particularly the cecum and appendix, were affected to a varying degree. The walls of appendix and cecum were thickened, edematous, and greatly congested. Very often more or less extended hemorrhages were associated with the edema. In the more severe cases superficial necrosis was observed (Fig. 5).

Heart.—The heart showed more or less hyperemia in the muscular wall. In some areas red blood corpuscles were found outside the capillaries (Fig. 6).

Liver.—In some cases we found distinct parenchymatous degeneration of the liver cells. In other cases this degeneration was slight, the peripheral cells of the lobules having a lighter color than the central ones. In a few cases no degeneration at all was noted. Hyperemia was always present, however (Fig. 7).

Kidneys.—The cells in the tubuli contorti were always degenerated. In a few cases we found regular parenchymatous degeneration, the nuclei being indistinctly stained. Usually the protoplasm of these cells appeared unstained, so that they acquired an appearance somewhat similar to fatty degeneration. Hyperemia was observed in the kidneys (Fig. 8).

The anatomical lesions described above were found in rabbits injected with exotoxin and endotoxin as well. Furthermore, it made no difference whether the toxins were derived from S, R, or R_n strains. It was noteworthy that the endotoxin, heated to 80°C. for 1 hour, caused the same anatomical changes in the spinal cord as the exotoxin.

DISCUSSION

We have demonstrated that the three serologically different variants of the Shiga bacillus, S, R, and R_n, are equally toxic and are able to cause the same lesions in rabbits. We must therefore conclude that the poisonous product does not depend upon the antigenic behavior of the types. Hence the toxins may be exotoxic in nature, produced by the living bacterial cells, or they may be endotoxins, derived from a part of the bacterial bodies, being similar in the three variants. The last supposition seems to be the most reasonable, as the toxin can be obtained by autolysis of the bacteria. It may, on the other hand, be

presumed that the Shiga bacilli, during their growth on agar surface, are able to secrete the toxin, which will go into solution, when the bacteria are emulsified in saline. Selter (8) has shown, however, that the dead bacterial bodies, which had been used in producing endotoxin, were still toxic after autolysis. Furthermore he succeeded in extracting the same toxin several times from the dead bacilli. There is therefore reason to believe that the toxins of the Shiga bacillus are derived from the bacterial bodies themselves, being thus endotoxic in nature.

Finally the question arises of the explanation for the great disagreement between our results and those of Olitsky and Kligler. Table III shows that the two rabbits injected with 0.032 and 0.016 cc. of the exotoxin exhibited symptoms referable to the nervous system only. It might be concluded that we are here dealing with the pure neurotoxin of Olitsky and Kligler. However, the second and third rabbit of Table III, injected with 1 cc. and 0.125 cc. respectively, showed, on the other hand, diarrhea and paralysis, and the anatomical changes in the intestines were important. The fourth rabbit, receiving 0.063 cc., had paralysis but no diarrhea. At autopsy the lesions in the intestines were distinct but not important. The small doses thus seem to injure the intestinal tract to a slight degree, while the large doses cause more severe changes there. The same conditions appear in Table I also, and in all our experiments the small amounts of toxin seem to have a slight effect only upon the intestines. We might therefore assume that our small doses correspond to the exotoxin (neurotoxin) of Olitsky and Kligler, and our large doses to the endotoxin (enterotoxin) of the same authors. This explanation cannot be said to give entire satisfaction, especially when we keep in mind the results of our anatomical studies. We are in complete disagreement with the authors mentioned as regards the outcome of neutralization tests.

An important change takes place in the toxin on heating to 80°C. for 1 hour. Its poisonous effect is diminished to a large degree, whereas its immunizing ability is unaltered. This temperature effect may possibly be of practical use.

As regards the anatomical lesions in the spinal cord and large intestine, we are in full agreement with the statements of Vaillard and Dopfer. The important changes in the small intestines which we have demonstrated, have previously had but little attention. The degen-

erative processes in the parenchymatous organs are worthy of remark. The liver lesions were rather small and inconstant but in the kidneys, on the other hand, degeneration was a constant phenomenon. There is reason to believe that the marasmus and prostration which the rabbits show after the injection of Shiga toxin are partly due to these changes in the kidneys and the liver. On the whole, the rôle of the kidneys in the course of dysentery should have greater attention. In 1923, Hanssen (17) demonstrated that there was a distinct rise in the total amount of urea in the blood, following regular and uncomplicated cases of dysentery caused by Type III (the Sonne bacillus). A chemical control of this injuring effect upon the kidneys in the toxin experiments, should prove of special interest. Furthermore one should test the effect of heat (80°C. for 1 hour) upon this injuring ability of the toxin.

The toxin of the Shiga dysentery bacillus evidently causes degenerative changes in several organs of rabbits. The lesions are accompanied by hyperemia and sometimes by hemorrhages. In the large intestine the hemorrhages often play an important rôle and dominate the anatomical picture. In other organs the hemorrhages are inconstant and less extensive, while hyperemia is observed often.

CONCLUSION

1. The S, R, and R_n variants of the Shiga bacillus are equally toxic.
2. The effect of the toxin upon rabbits is the same, whether it is derived from filtrates of broth cultures (3 to 6 days old), or is obtained by autolysis of the killed bacteria, grown on agar surface. Rabbits show in both cases prostration, loss in weight, paralysis, and diarrhea.
3. When the toxin is heated to 80°C. for 1 hour, its poisonous effect nearly disappears, but its immunizing ability is unaltered. This heated toxin induces a formation of antitoxin, which can protect against the unheated toxins.
4. The anatomical changes observed in the spinal cord (degeneration of the motor neurons) and in the cecum (hyperemia and hemorrhages) are in agreement with the statements of previous authors. Furthermore, the toxin causes hyperemia and hemorrhages in the heart, hyperemia and degeneration in the kidneys and the liver.

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EXPLANATION OF PLATES

PLATE 1

FIG. 1. Rabbit 1-41 injected intravenously with 2 cc. S exotoxin. Died after 6 hours. The section from upper thoracic region of the spinal cord shows the swollen neurons with the large Nissl bodies.

FIG. 2. Rabbit 1-08 injected intravenously with 0.125 cc. S endotoxin (not heated). Died after 30 hours, symptoms: diarrhea and paralysis. The section is from the lower thoracic region of the medulla. Small, sclerotic, and homogeneously colored cells are recognizable.

FIG. 3. Rabbit 1-11 injected intravenously with 0.016 cc. S endotoxin (not heated). Died after 60 hours, symptoms: diarrhea and paralysis. The section from the upper lumbar region of the medulla shows the degenerated cells and necrotic areas.

FIG. 4. Rabbit 1-13 injected intravenously with 0.25 cc. R endotoxin (not heated). Died after 20 hours. Section from small intestine. The necrosis of the superficial cells is evident. Several leucocytes have invaded the mucous membrane, and the glandular elements are destroyed.

PLATE 2

FIG. 5. Rabbit 1-38 injected intravenously with 0.002 cc. S endotoxin (not heated). Diarrhea not observed, but paralysis of the extremities. Died after 60 hours. The section from cecum demonstrates hemorrhages and hyperemia in the mucosa, necrosis of the superficial cells. Considerable edema in the sub-mucosa.

FIG. 6. Rabbit 2-87 injected intravenously with 4 cc. R exotoxin. Died after 20 hours. The section from the heart shows the marked hyperemia in the muscular wall.

FIG. 7. Rabbit 1-35 injected intravenously with 1 cc. R endotoxin (not heated). Died after 12 hours. The section from the liver demonstrates that some of the cells (the peripheral) are lighter colored than the others.

FIG. 8. Rabbit 1-35. The section from the kidney shows the peculiar appearance of the cells in tubuli contorti. In most of the cells the protoplasm is unstained.

(Waler: Toxin production of Shiga bacilli)

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