# INITIAL STUDIES ON THE TOXINS OF *SHIGELLA DYSENTERIAE* TYPE 1 AND *S. FLEXNERI*

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The production of an exotoxin from *Shigella dysenteriae* type 1 was described as early as 1903 [1]. The exotoxin was called Shiga neurotoxin because the cell free media supernatants from this organism caused an illness of the central nervous system along with limb paralysis and subsequently caused death. Olitsky and Kligler demonstrated that the toxin preparation contained two biologically active compounds, one being the lipopolysaccharide cell wall material and the other a protein [2].

It was observed that the organisms themselves did not elaborate a neurotoxic response whereas the cell free supernatants did. It was not until Keusch et al described the production of an enterotoxin in 1972 that there was renewed interest in the toxins elaborated by *S. dysenteriae* type 1 [3,4]. The material was found to be cytotoxic and was assayed in He La cell monolayers [5]. The relationship of Shiga enterotoxin-neurotoxin and cytotoxin was investigated by Keusch and Jacewicz [6]. Their studies suggest that the same molecule is active as neurotoxin and cytotoxin. However, cytotoxicity was demonstrated by two separate fractions, only one of which contained the neurotoxic and enterotoxic properties. Enterotoxins from *Vibrio cholerae* and *Escherichia coli* were not cytotoxic as measured by the He La cell assay and thus is different from *Shigella dysenteriae* type 1 toxin [7]. While much work has been done with regards to the enterotoxin produced by *Shigella dysenteriae* type 1, other *Shigella* species have not been studied extensively for enterotoxin producing capability. Dysentery caused by *Shigella flexneri* is on the increase in comparison to *Shigella dysenteriae* type 1. I have carried out some studies with the enterotoxin producing capability of *Shigella flexneri*. The virulent strains of *Shigella flexneri* has been demonstrated to produce Keratoconjunctivitis in the eyes of the rabbits and guinea pigs [8].

Ability to cause Keratoconjunctivitis was also shown to be correlated with the ability of *S. flexneri* cells to invade and multiply within He La cells. However, no strain of *Shigella flexneri* has been demonstrated to be producing enterotoxin as measured in the mammalian small intestine. In this paper I shall discuss the toxin producing ability in vitro, by a number of selected *Shigella dysenteriae* type 1 (Shiga) and *S. flexneri* (Flex), as measured by fluid accumulation in the ligated small intestine of Rabbits.

## Materials and Methods

Shiga and Flex isolates from heavy purgers admitted in the Cholera Research Laboratory Hospital were used for starting inocula for shake cultures [9]. 100 ml casamino acid culture media were used in 500 ml erlenmeyer flasks and was incubated at 30° + 1°C for 18 hours. The culture suspension was centrifuged at 10,000 r.p.m. at 0°C for 30 minutes. The cells were discarded and the supernatant was first passed through .45 μ and then through .22 μ millipore filter. Passage of the supernatants through these filters were more difficult than passing Vibrio cholerae culture filtrates. The sterile preparation was stored at -30 to -40° C.

For the detection of the presence of toxin young adult rabbits were used essentially following previously described techniques [10, 11]. In short laparotomy was performed on rabbits anesthetized with Sodium Pentobarbital, the small intestine washed with 20 ml isotonic saline and 6 ligated loops made, each approximately 10 -12 cm long. Each rabbit was challenged with a cholera toxin positive control and an isotonic saline negative control. For screening purposes 5 ml crude culture filtrate was injected in each loop, and two rabbits were used in each set.

18 hrs. later the fluid in the loops and loop length was noted. Fluid per cm of loop (V/L) was calculated from this data. Rabbits that did not accumulate fluid in the loop with cholera toxin or yielded positive loops with isotonic saline were not included in the data. If the response with 5 ml challenge dose was good the same crude filtrate was tested in 3 or more loops with 2 ml challenge doses.

He La cell assay was carried out by counting the number of cells degenerated after adding a concentrated Shiga toxin preparation in various dilutions. Crude culture filtrates were also tested in the skin of the back of adult rabbits by the method described by Craig [12, 13]. For testing short term skin permeability the Rabbits were first injected with Pontamine Sky Blue and then the crude filtrates injected in 0.1 ml volumes.

## RESULTS

Crude culture filtrates from fifty-two selected strains of S.dysenteriae type 1 were tested in the ligated intestinal loop of rabbits. Table 1 shows that there were 15 shiga culture filtrates that produced 2 - 2.5 ml fluid per cm of loop, 27 filtrates with 1 - 1.9 ml/cm, 8 with 0.2 - 0.9 ml/cm and 2 with no fluid accumulation in the loop when 5 ml challenge doses were used per loop. Selected strains from the upper V/L range with 2 ml challenge dose did not have any loop producing 2 or more ml/cm, 2 produced 1 - 1.9 ml/cm and 22 produced 0.2 - 0.9 ml/cm. Table 2 shows the enterotoxicity of Shigella

flexneri crude culture filtrates as tested in the ligated small intestine loop of rabbits. There was only one strain with 2.1 ml/cm of loop, 7 with 1 - 1.9 ml/cm, 9 with 0.2 - 0.9 ml/cm and 23 with no fluid accumulation when 5 ml challenge dose was used per loop. None of the filtrates used with 2 ml challenge doses per loop accumulated any fluid. Table 3 shows the comparison of Shiga and Flex crude culture filtrates. In this table, I included only those cases which produced more than 1 ml of fluid per cm of loop when 5 ml challenge doses were used. 77% of the Shiga and 20% of the Flex filtrates were positive on 1 ml or more fluid/cm basis.

Figure 1 shows the number of He La cells degenerated when a 100 fold concentrated shiga toxin was used at various dilutions. The crude filtrate was cytotoxic, as apparent from the degeneration of cells with 1/400 th ml of the toxin preparation. NIH Lot 1 cholera toxin was used as control in this assay and was found to have no cytotoxic effect on the He La cells.

The results of the injections in the skin of the back of adult rabbits was uniformly negative for both Shiga and Flex preparations when observed at 24 hours. Cholera toxin was used as control and yielded positive bluing. When the Pontamine Sky Blue was injected first and then the intracutaneous injections, there was no immediate bluing by any of the toxin preparations tested. At about 15 minutes there was a transient blue appearance with the Flex preparation and none with Shiga or cholera toxin, I have made these observations only twice.

## Discussion

In the context of a Shigellosis epidemic predominantly caused by Shigella flexneri it is important to know whether these organisms elaborate an enterotoxin. In vitro toxin production cannot be taken as an indicator of virulence of Shigella organisms in vivo.

Variability in the amount of fluid produced in the ligated ileal loop of rabbits is well known. I am planning to test some of these crude filtrates in more rabbits so that an average of at least 3 rabbits can be obtained. I plan to culture the organism at 35° and 37° C to see if there is any increase in the toxin production. Crude culture filtrates from other Shigella spp. would also be assayed in the rabbit loop model. However, I plan to concentrate my efforts on the toxin obtained from Shigella dysenteriae type 1 and S. flexneri. Studies are planned to compare the properties of the two toxin preparations.

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TABLE 1

## ENTEROTOXICITY OF *SHIGELLA DYSENTERIAE* TYPE 1 CRUDE CULTURE FILTRATES

LIGATED SMALL INTESTINE LOOP ASSAY IN RABBITS

| RANGE OF V/L * | 5 ML/LOOP | 2 ML/LOOP |
|---|---|---|
| 2.0 - 2.5 | 15 | 0 |
| 1.0 - 1.9 | 27 | 2 |
| 0.2 - 0.9 | 8 | 22 |
| 0 | 2 | 0 |

* V/L = VOLUME OF FLUID PER CM OF LOOP

TABLE 2

ENTEROTOXICITY OF SHIGELLA FLEXNERI CRUDE CULTURE FILTRATES

LIGATED SMALL INTESTINE LOOP ASSAY IN RABBITS

| RANGE OF V/L * | 5 ML/LOOP | 2 ML/LOOP |
|---|---|---|
| 2.0 - 2.1 ML | 1 | 0 |
| 1.0 - 1.9 ML | 7 | 0 |
| 0.2 - 0.9 ML | 9 | 0 |
| 0 | 23 | - |

*V/L = VOLUME OF FLUID PER CM OF LOOP

TABLE 3

COMPARISON OF S. DYSENTERIAE TYPE 1 (SHIGA) AND S. FLEXNERI (FLEX) CRUDE CULTURE FILTRATES

LIGATED SMALL INTESTINE LOOP ASSAY IN RABBITS

| SPECIES | TOTAL NO TESTED | # POSITIVE* | %POSITIVE |
|---|---|---|---|
| SHIGA | 52 | 42 | 77% |
| FLEX | 40 | 8 | 20% |

* 5 ML CHALLENGE DOSE PRODUCING MORE THAN 1 ML FLUID/CM OF LOOP (# POSITIVE)

FIG 1

EFFECT OF SHIGELLA DYSENTERIAE TYPE 1 ON HE LA CELLS