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ENTEROTOXIC ACTIVITY OF LIVING CULTURES OF *Shigella sonnei* AND ENTEROTOXIN FORMATION *in vivo*

N. B. Kamzolkina, I. L. Gofman, I. L. Margulis, L. M. Yarovaya, and I. N. Kamenskaya

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The overwhelming majority of virulent strains of Shigella sonnei caused the accumulation of fluid in the lumen of an isolated segment of rabbit small intestine; the fluid contained large quantities of mucus and sometimes blood; the mucous membrane of the segment was hyperemic and had petechial hemorrhages. Avirulent strains of Sh. sonnei as a rule did not cause exudation into the loop of intestine. The sterile and concentrated contents of the intestinal loops of rabbits responding to injection of the virulent strain of Sh. sonnei or a toxigenic strain of Shigella shigae invariably gave a positive reaction in other rabbits. The character of the exudate and the changes in the mucous membrane under these circumstances were indistinguishable from those following injection of living cultures.

KEY WORDS: Shigella sonnei; Shigella shigae; enterotoxin

Investigations have shown [7] that most recently isolated strains of Shigella sonnei can induce the accumulation of fluid in the isolated loop of rabbit small intestine. However, Floyd and Arm were unable to reproduce this phenomenon by injecting filtrates of these cultures, and Arm et al. [1] also were unsuccessful when injecting lysates obtained by ultrasonic treatment.

These workers observed correlation between the enterotoxicity of the strains and their virulence.

More recent data [2-7] indicate that Sh. sonnei, unlike Shigella shigae, is unable to produce an enterotoxin.

In the present investigation the isolated loop of rabbit small intestine was used as a model to study the action of recently isolated strains of Sh. sonnei and also of the sterile contents of isolated segments of intestine obtained during tests of virulent strains.

EXPERIMENTAL METHOD

All strains, which were generously provided by the bacteriological laboratory of the Leningrad District Public Health Station in Moscow (Head T. A. Lakshtanova), were kept in the lyophilized state at 4°C. Altogether 14 recently isolated strains and 2 reference strains (Nos. 9090 and 7478) were tested. The strains were grown in dishes on nutrient agar, the round colonies were chosen as far as possible, and a suspension prepared from them in physiological saline with a density of 1 billion bacterial cells/ml according to the optical standard of the State Control Institute, and 1 ml of this suspension was injected into the lumen of an isolated loop of rabbit small intestine.

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TABLE 1. Enterotoxicity of Living Cultures of Shigella sonnei

Culture	Biochemical type	Phase of microorganism	Per cent of segregation of phase II	Strain No.	Number of rabbits with positive reaction	Number of rabbits with negative reaction
Virulent	IIg	I, II, R	25	1041	9	
		I, II, R	10	3020	6	
		I, II	25	1681	2	
		I, II, R	25	9543	1	
		II, I	75	9413	1	1
		II, I, R	75	9560	2	
		II, I	85	9574		4
	III d	II, I	75	9571	2	
		II, I	65	9316	1	
Avirulent	II g	I, II	10	9090		4
		I, II	10	7478		1
		II, R	100	2953	1	
		II	100	12300		2
	III d	II, R	100	2716		2
		II	100	1326		1
		II, I, R	85	288	1*	1

*The culture was tested in a dose of 2 billion bacterial cells.

The presence of phase II in the cultures was determined by agglutination of the colonies on a slide with homologous serum, and also by the aid of specific phage. Phase R was identified by testing agglutination of the colonies on a slide with physiological saline.

The contents of the intestinal loops obtained immediately after sacrifice from rabbits reacting positively to injection of the most virulent strain No. 3020, and also of an R strain of Shigella shigae No. 973a (control) were centrifuged at 9000 rpm for 20 min to remove mucus, blood cells, and most of the microorganisms. The clarified supernatant was sterilized with chloroform vapor and checked by repeated subculture, after which it was freeze-dried.

Chinchilla or albino rabbits weighing 1.5-2 kg, deprived of food for 2 days, were used. Under ether anesthesia 6 to 8 segments of small intestine were isolated 20-30 cm above the appendix, each 6-10 cm long, and 3 or 4 cultures were injected into alternate segments. The following day the rabbits were killed (by air embolism) and the coefficient of dilatation determined (in ml/cm).

The virulence of the strains was tested by the kerato-conjunctival tests on guinea pigs.

EXPERIMENTAL RESULTS

The rabbits tolerated injection of more than two virulent cultures into the intestine badly: Of 21 animals 6 died during the first day and 1 developed paralysis of the hind limbs. As Table 1 shows, living cultures of some strains were tested on several rabbits. In this case, the result was the same in the living rabbits and those which died; the results of experiments on both groups of animals were accordingly considered together.

All virulent strains except No. 9574 gave the phenomenon of dilatation of the intestinal loop. One of the rabbits on which this strain was tested died, however, before the exudate had had time to accumulate in the ligated segment of intestine (control with living strain No. 1041, giving a coefficient of dilatation of 0.5 ml/cm). In the other two rabbits this coefficient was 0.5 and 0.6 ml/cm respectively (result doubtful). No control with a living culture was set up for these rabbits, for in the course of the work it was discovered that injection of virulent cultures of the microorganism reduced the likelihood of the rabbits' survival. Later, as positive control, a cholerogen kindly provided by Dr. L. T. Karaeva from the Saratov "Mikrob" Institute, was used. The cholerogen was injected into the most distal segment.

Some rabbits did not react to injection of the cholerogen or of living and known exterotoxic strains of Sh. sonnei or Sh. Shigae. Some of them, as was found during the operation, suffered from cysticercosis. The albino rabbits were more sensitive to the enterotoxic action of the cholerogen and living cultures.

As Table 1 shows, virulent strains as a rule gave a positive result on all rabbits on which they were tested (for example, strains Nos. 1041 and 3020). Only one weakly virulent strain (No. 9413), predominantly in phase II, caused exudation in 1 of 2 rabbits.

The ability of a culture of strain No. 1041, suspended in physiological saline, to induce dilatation of the segment of intestine, persisted after standing for 3 days at 4°C (period of observation).

Different rabbits reacted differently to injection of the same virulent culture. In the overwhelming majority of cases the serous effusion contained much mucus, at times mixed with more or less blood. The mucous membrane of the intestine, dilated by exudate, was always very hyperemic, and it often had petechial hemorrhages.

Two virulent strains were titrated in a loop of rabbit intestine. The minimal enterotoxic dose for strain No. 1041 was 31 million, and for strain No. 3020 it was 125 million bacterial cells.

Of 7 avirulent cultures only 2 recently isolated strains (Nos. 2953 and 288), which had lost their virulence during subculture and freeze-drying, caused the formation of an exudate (strain No. 288 in a dose of 2 billion bacterial cells). The remaining avirulent strains constantly gave a negative result.

Tests of two strains (Nos. 9090 and 12300) in a dose of 8 billion bacterial cells per rabbit gave a negative result despite a positive response to the virulent strain.

Belonging to different biochemical types did not affect the virulence or enterotoxicity of the strains. Although nearly all avirulent strains were predominantly in phase II, segregation of colonies of one phase or another by them was not by itself responsible for the properties mentioned above. For instance, avirulent strains Nos. 9090 and 7478, like the virulent strains, formed colonies in phase I, whereas virulent and enterotoxic strains Nos. 9413 and 9560 consisted chiefly of microorganisms in phase II.

The virulence of strains of Sh. sonnei correlated more with their enterotoxicity than with the phase state of the cultures.

Repeated testing of the enterotoxic activity of the sterile liquid contents of the loops of rabbit intestine formed in response to injections of strain No. 3020 gave positive results in isolated cases when not less than 5 ml was injected. A positive reaction was constantly observed if concentrated preparations equivalent to 15 ml of liquid contents and obtained either from rabbits infected with Sh. sonnei or from rabbits infected intrasegmentally by a culture of Sh. shigae, were used. The contents of intestinal loops of rabbits reacting to these concentrated preparations were indistinguishable in character from those formed in response to injection of living dystentery strains (mucus, blood, hyperemia, and hemorrhages into the mucous membrane).

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