WATER FLEAS AS BIOLOGICAL ANTAGONISTS OF NAG VIBRIOS

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The increased frequency of intestinal infections in recent years is linked with increased microbial contamination of water supplies which, under certain conditions, may become a source of infection. The greatest risk as regards the spread of diseases caused by different species of vibrios is the water of open reservoirs, in which vibrios can remain for a long time and even propagate [9]. It is not by accident that by resolution of the World Health Organization the period from 1980 to 1990 is declared to be the International Decade for Water Supplies and Sanitation.

The lower crustaceans, which are widely represented among the inhabitants of natural reservoirs, notably water fleas of the genus *Daphnia*, are known to feed on debris, algae, and bacteria nonpathogenic for man [1-8]. It was accordingly considered interesting to discover whether water fleas can feed on enteropathogenic NAG vibrios.

The object of this investigation was to obtain a stable laboratory culture of *Daphnia magna straus* and to study relations between the water fleas and enteropathogenic strains of NAG vibrios. Bacteriological and luminescence-serologic methods were used.

EXPERIMENTAL METHOD

A laboratory culture of *Daphnia magna straus* was obtained by multistage adaptation. Up to 10,000 water fleas (females, males, young) were used in the series of experiments and were infected with five museum strains of NAG vibrios isolated from the feces of patients, and one strain isolated from an open reservoir in 1970.

In the experiments of series I the viability of the water fleas was studied under different conditions of feeding. For this purpose, 30 water fleas were placed in each of a number of sterile flasks with sterile tap water and distributed in groups depending on the experimental conditions: The water fleas of group 1 were fed a living culture of NAG vibrios; those of group 2 were fed with a culture of heat-killed NAG vibrios; those of group 3 received a suspension of a living culture of NAG vibrios and *Chlorella*, at the rate of 5 million bacterial cells per water flea per day. Water fleas kept under similar conditions and fed with *Chlorella* only (group 4) and fleas kept under conditions of completed starvation (group 5) served as the controls. The experimental and control water fleas were kept at a temperature of 20-24°C (Table 1).

In the experiments of series II the number of NAG vibrios ingested was estimated quantitatively. The water fleas were kept under the same conditions as in series I and were fed with different cultures of NAG vibrios. The same cultures of NAG vibrios in water, in the same numbers, but without water fleas, served as the control. Every day bacteriological seedings were taken of water from the experimental and control vessels on differential diagnostic media: TCBS medium, alkaline agar, Endo's agar, and peptone broth. Homogenates of water fleas were tested periodically by bacteriological methods for the presence of vibrios.

The experiments of series III yielded practically "sterile" water fleas. As a first step, to identify the microflora of the water fleas their homogenates were seeded on differential-diagnostic media: nutrient agar, Endo's agar, and Levine's medium. The sensitivity of the microflora of the water fleas was also tested by means of a dish method using disks, to various antibiotics: tetracycline, oleandomycin, polymixin, erythromycin, and ristomycin.

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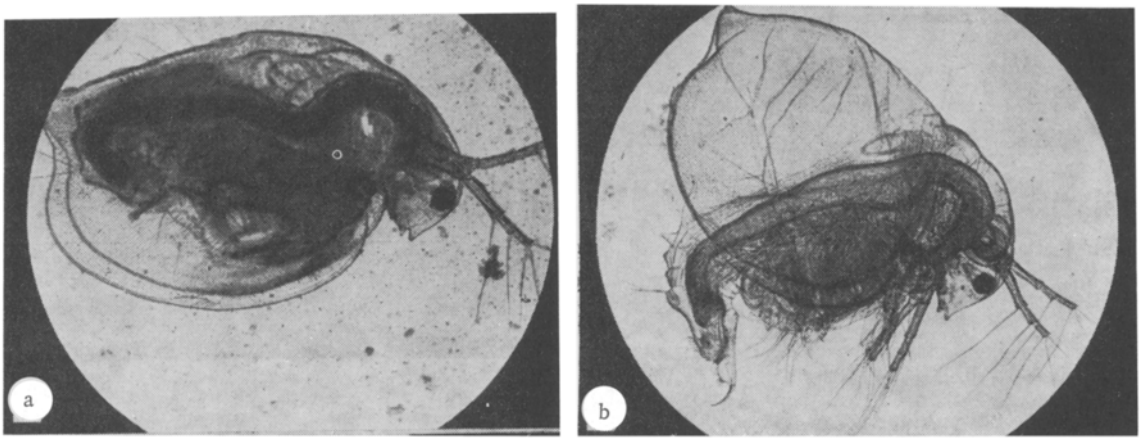

Fig. 1. Intestine of water flea filling with culture of NAG vibrios: a) experiment, b) control.

TABLE 1. Distribution of Water Fleas into Groups Depending on Experimental Conditions

Group of water fleas	Conditions of keeping and diet	Number of water fleas	Sex and age	Viability, days
1-	Living culture of NAG vibrios	600	♀	12
			♂	18
			M	27
2-	Heat-killed culture of NAG vibrios	600	♀	15
			♂	19
			M	28
3-	Suspension of living culture of NAG vibrios and Chlorella	600	♀	38
			♂	29
			M	35
4-	Chlorella (control)	600	♀	33
			♂	25
			M	34
5-	Starvation (control)	600	♀	1—3
			♂	1—2
			M	1—2

Legend. M) Young fleas.

The water fleas were placed 30 at a time in sterile flasks with 200 ml of sterile tap water and were fed on sterile *Chlorella*. Tetracycline was added to the water in a dose of 5 i.u./ml. The water fleas were washed daily with sterile water and transferred from one vessel to another containing the same concentration of antibiotics. Bacteriological seedings were taken after 3-5 days from the water and homogenates of the water fleas on Endo's and Levine's media, nutrient agar, and Hottinger's broth. After 28-30 days, no microorganisms could be grown from water of the experimental vessels or from the water fleas contained in them. A quantitative estimation of NAG vibrios ingested was again carried out on the practically "sterile" water fleas, as in the previous experiment.

Water fleas were studied by a luminescence-serologic method at various times after contact with NAG vibrios. The water fleas were fixed in toto in 4% Lillie's buffered formalin and embedded in paraffin wax. Serial sections were subjected to parallel histologic study by Coons' indirect method. To identify the bacteria by this method, specific sera obtained in the writers' laboratory against cultures of NAG vibrios were used. The necessary controls for specificity of luminescence were set up.

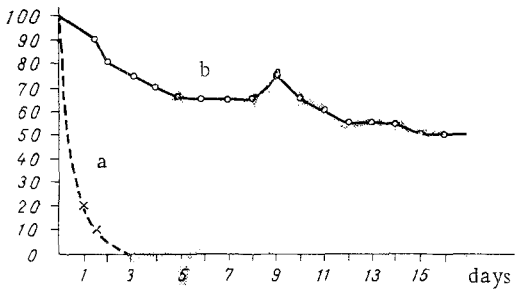

Fig. 2

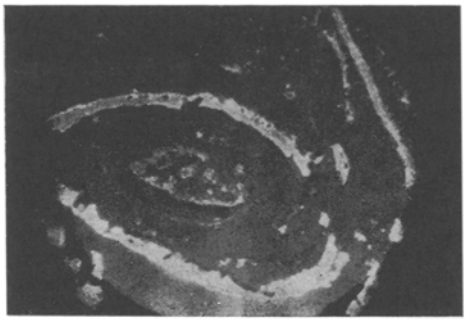

Fig. 3

Fig. 2. Time course of results of feeding NAG vibrios: a) from water with water fleas; b) from water of control vessel (NAG vibrios in water without water fleas). Abscissa, time after feeding (in days); ordinate, percentage of successful feeding of NAG vibrios.

Fig. 3. Middle portion of intestine of water flea 24 h after feeding with NAG vibrios. Specific luminescence of brush border and cytoplasm of cells of intestinal epithelium, 120×. Indirect Coons' method.

EXPERIMENTAL RESULTS

Relations between the water fleas and the different cultures of NAG vibrios were judged from their behavior, their ability to multiply, and their viability. After introduction of the water fleas into the experimental flasks they began to filter and swallow the food. When a hanging drop containing a water flea and vibrios was studied under the microscope it was found that during the first 10 min a food ball was formed in front of the water flea's mouth, and passed into the esophagus. During the next 30-40 min food masses were found in various parts of the intestine, and portions of feces were excreted (Fig. 1). Visual observation of the behavior of the water fleas showed that it was normal in the experimental vessels, the females produced a progeny, and the young fleas developed into adults. The experimental water fleas lived between 12 and 38 days depending on the experimental conditions, but control fleas kept under conditions of starvation lived only 1 to 3 days (Table 1).

The results of these investigations show that water fleas swallow NAG vibrios and feed on them. This was also confirmed by the phenomenon of clarification of the microbial suspension after contact with the water fleas. No NAG vibrios could be seeded from this clarified water. Similar results were obtained after contact between water fleas and suspensions of salmonellas, shigellas, and yersinias.

In the next series of experiments the number of vibrios swallowed was counted. As Fig. 2 shows, the number of vibrios in water with water fleas diminished daily and by the 3rd day no vibrios could be grown from seedings, whereas in water without water fleas, but with the same load of vibrios (control), the number of microorganisms remained almost unchanged until th 10th-24th day of observation.

Since the water flea's own microflora periodically grew on the differential diagnostic media, which interfered with counting the colonies of vibrios, it was decided that steps should be taken to obtain "practically sterile" crustaceans. For a controlled study of the feeding of water fleas on NAG vibrios, experiments also were carried out with "germ-free" water fleas of the species *Daphnia magna straus*.* To inhibit the microflora of the water fleas a broad-spectrum antibiotic (tetracycline) to which their microflora was highly sensitive, was used. Experiments with germ free water fleas confirmed the results of the previous investigations.

It was shown by the luminescence-serologic method that during the first 30-60 min after the beginning of introduction of bacterial cultures into the water containing the water fleas, luminescent vibrios were seen in the intestine of the crustaceans. The number of vibrios was smaller after 3 h, and after 24 h only single microorganisms were found in the lumen of the water fleas' intestine. At the same time of the experiment, bright specific luminescence of the brush border and cytoplasm of cells of the intestinal epithelium was

*No bacteriological investigations of anaerobic microflora were undertaken.

found only in the middle portions of the intestine of the water fleas, where absorption of the luminescent components of the products of digestion of the vibrios evidently takes place (Fig. 3).

The results of these experiments thus showed for the first time that water fleas feed on enteropathogenic cultures of NAG vibrios, digest them, and assimilate this food with the aid of their digestive enzymes. Water fleas, which inhabit reservoirs in enormous numbers, participate in their purification and play the role of biological scavengers. This property of these organisms can be utilized to good purpose by the antiepidemic and hygiene services for the prevention and control of intestinal infections.

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PERCENTAGE OF EAC-RFC AND T_γ CELLS IN THE THYMUS AND SPLEEN OF HUMAN FETUSES

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The present stage of development of immunology is characterized by a penetrating and all-embracing study of receptors of immunocompetent cells (ICC), i.e., those unique structural formations by means of which the numerous cooperative interactions leading to different types of immunologic reactions take place [3, 4, 11,]4]. There have been many studies of the formation of receptors to ICC in the prenatal development of bone marrow [1, 6-8, 14, 15] and various animals [3, 9, 10]. Nevertheless, analysis of some of them [13] reveals considerable technical errors, which have led to the obtaining of incorrect data and, as a result of this, to an incorrect interpretation of the true state of affairs. The problem of formation of certain receptors such as, for example, the receptor for the Fc fragment of IgC on T lymphocytes of human fetuses is not mentioned at all in the literature, although the property of T suppressors has been ascribed to lymphocytes of this type [12].

In the investigation described below the percentages of EAC rosette-forming cells (EAC-RFC) and of T_γ cells were studied in the thymus and spleens of human fetuses.

EXPERIMENTAL METHOD

Altogether 22 human fetuses aged 11-28 weeks, obtained as a result of spontaneous abortions from clinically healthy mothers not receiving any drugs were used. In two cases the abortions were criminal. Four suspensions from the thymus and spleen were obtained by the method described by Raitsina et al. [5]. The T lymphocytes were obtained from the spleen by the

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