Daphnia magna STRAUS AS A CONSUMER OF ENTEROPATHOGENIC BACTERIA

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Despite much progress in reducing the prevalence of infectious diseases, the problem of intestinal infections remains acute. In recent years great importance in the spread of intestinal diseases has been attached to water of open reservoirs, in which enteropathogenic bacteria remain viable, and in some cases may actually multiply, for a long time [2-15]

The writers showed previously that lower crustaceans — suborder Cladocera, family Daphniidae (Straus), genus *Daphnia* O. G. Müller, species *Daphnia magna* Straus — which are widespread in nature, can utilize enteropathogenic strains of NAG vibrios as food [1].

The aim of this investigation was to study the ability of *Daphnia magna* to utilize as food other enteropathogenic bacteria and, in particular, salmonellas, shigellas, and yersinias. Bacteriological and radionuclide methods of investigation were used to study these problems.

EXPERIMENTAL METHOD

Up to 15,000 daphnias were used in two series of experiments. Strains of enteropathogenic bacteria were obtained from the L. A. Tarasevich State Research Institute for Standarization and Control of Medical Biological Preparations and from the Central Research Institute of Epidemiology (the collection of Dr. H. H. Mollaret, Institut Pasteur, Paris). In the experiments of series I the crustaceans were placed in vessels with sterile water into which suspensions of the bacteria *Salmonella enteritidis, S. typhimurium, Shigella sonnei, Sh. flexneri, Yersinia pseudotuberculosis* C-1, and *Y. enterocolitica* C-9 were added as food at the rate of 5 to 10 million bacterial cells per daphnia per day. Daphnias kept under identical conditions but recieving chlorella and yeast cells as food, and suspensions of the corresponding bacteria in flasks of sterile water in the same number, but without daphnias, served as the controls. The daphnias were observed daily, and bacteriological seedings of water from the experimental and control vessels on Endo's, Levin's, and Ploskirev's differential-diagnostic media were made every 1-2 days throughout the experiment. Homogenates of daphnias were tested periodically for the presence of enteropathogenic bacteria. Since the endogenous microflora of daphnias grew periodically on Endo's and Levin's media, and this interfered with the exact counting of colonies of enteropathogenic bacteria which had grown, the method of radionuclide labeling of the bacteria was adopted.

In the experiments of series II these same enteropathogenic bacteria but labeled with radionuclides were used as food. [^{14}C]Glucose (200 μCi/liter, specific radioactivity 50 mCi/mmole) and tritium-labeled proline and leucine (833 μCi/liter and 2.5 mCi/mmole; 350 mCi/liter and 89.2 mCi/mmole respectively) were added to the media on which the bacteria were grown. Seedings were incubated at 37°C for 18-20 h. Cultures which grew were washed off with physiological saline, and then washed three times with physiological saline in a centrifuge in the cold. The bacterial suspension, in a concentration of 10 billion bacterial cells/ml, was treated three times with cold 5% TCA and twice with cold absolute alcohol. The acid-soluble fraction was deposited on millipore filters and dried. The degree of labeling of the microorganisms was determined on a Packard Tricab scintillation counter. The daphnias were distributed into two groups depending on the experimental conditions. Crustaceans of group 1 were placed in vessels with sterile water and fed on the above-mentioned radionuclide-

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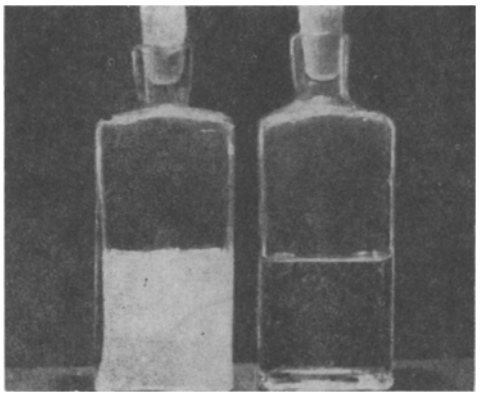

Fig. 1. Clearing of microbial suspension in vessel with daphnias. On left — control, on right — experiment.

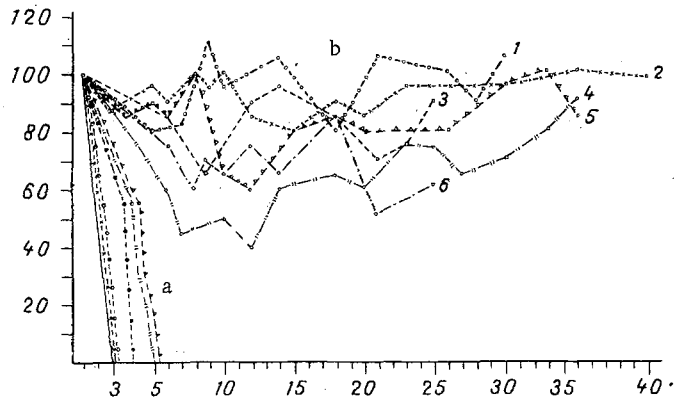

Fig. 2

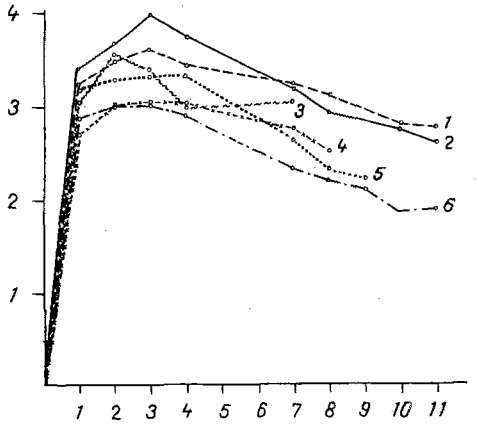

Fig. 3

Fig. 2. Time course of positive seedings of enteropathogenic bacteria. Abscissa, time after feeding (in days); ordinate, positive seedings of enteropathogenic bacteria (in %). a) From water with daphnias; b) from water of control flasks (enteropathogenic bacteria in water without daphnias). 1) *S. enteritidis*, 2) *S. typhimurium*, 3) *Y. enterocolitica*, 4) *Sh. flexneri*, 5) *Sh. sonnei*, 6) *Y. pseudotuberculosis*.

Fig. 3. Time course of feeding of daphnias on enteropathogenic bacteria labeled with [^{3}H]glucose. Abscissa, time (in days); ordinate, logarithm of number (cpm). 1) *S. enteritidis*, 2) *S. typhimurium*, 3) *S. flexneri*, 4) *S. sonnei*, 5) *Y. enterocolitica*, 6) *Y. pseudotuberculosis*.

labeled enteropathogenic bacteria. To determine the intensity of accumulation of the label in the crustaceans, samples of daphnias and water from each flask were taken every 1-2 days throughout the experiment. The daphnias were washed with water, homogenized, and radiactivity in the bodies of the crustaceans and in the water was assayed. The dynamics of accumulation of labeled bacteria in the crustaceans and of excretion of their breakdown products was studied in the daphnias of group 2. The daphnias were placed in flasks with sterile water and fed on labeled enteropathogenic bacteria (like the crustaceans of group 1) for 3 days, after which the daphnias were carefully washed with water, transferred to other flasks with water, and fed on unlabeled chlorellas and yeast cells. To determine the content of labeled breakdown products of bacteria, samples of daphnias and water were taken daily.

EXPERIMENTAL RESULTS

Visual observation of the behavior of the daphnias in the experimental and control vessels revealed no evident differences. After 3-5 days complete clearing of the microbial suspension was observed in the experimental vessels in which the daphnias fed on enteropatho-

genic bacteria could be seeded. In the control vessels containing the same quantity of microbial suspension of the above-mentioned bacteria in sterile water, but without daphnias, the water remained just as turbid throughout the experiment (Fig. 1). Seedings of water from the control vessels yielded colonies of enteropathogenic bacteria throughout the experiment; their number, moreover, either fell negligibly or increased on account of reproduction (Fig. 2). The level of radioactivity after feeding the daphnias with labeled bacteria reached a maximum after 2-3 days and then fell, and remained at about the same level until the end of the experiment (Fig. 3). Excretion of breakdown products of enteropathogenic bacteria labeled with glucose took place more rapidly than in the case of those labeled with choline. Throughout the period of observation the daphnias assimilated breakdown products of these enteropathogenic bacteria actively.

These experiments showed for the first time that daphnias utilize enteropathogenic bacteria (salmonellas, shigellas, yersinias) as food. The absence of narrow specificity toward any particular species of microorganisms and the wide geographic distribution of daphnias are evidence of the important role of lower crustaceans in biological self-purification of reservoirs. Controlled culture of daphnias in water catchment areas can be used to maintain the optimal state of hygiene in reservoirs.

LITERATURE CITED

1. A. P. Avtsyn, V. A. Shakhlamov, R. S. Trager, et al., Byull. Éksp. Biol. Med., No. 1, 48 (1982).
2. É. M. Aminadze, K. A. Artykov, R. A. Amanova, et al., in: Problems in Prevention of Virus Hepatitis and Bacterial Infections [in Russian], Tashkent (1980), pp. 144-146.
3. G. A. Bagdasar'yan, Yu. G. Talaeva, A. N. Zakharkina, et al., Gig. Sanit., No. 7, 59 (1980).
4. P. N. Burgasov and G. I. Sidorenko, Vestn. Akad. Med. Nauk SSSR, No. 3, 3 (1981).
5. V. V. Vlodavets and G. P. Kashina, Zh. Mikrobiol., No. 12, 33 (1977).
6. E. N. Merkina, N. B. Dalechin, F. Kh. Ibragimov, et al., in: Current Problems in Epidemiology and Infectious Diseases [in Russian], Saratov (1976), pp. 26-28.
7. "WHO Scientific Working Group," WHO Bulletin (Russian edition), 58, 274 (1980).
8. V. I. Nemyrya and M. D. Bogatireva, Gig. Sanit., No. 3, 77 (1980).
9. V. I. Nemyrya, V. V. Kolesnikova, and Yu. N. Nikitina, in: Current Problems in Hygiene and Occupational Pathology under Conditions of Scientific and Technical Progress [in Russian], Tashkent (1980), pp. 54-56.
10. T. A. Nikolaeva, G. A. Bagdasar'yan, A. I. Tsikova, et al., Vestn. Akad. Med. Nauk SSSR, No. 3, 46 (1975).
11. G. I. Sidorenko, G. A. Bagdasar'yan, Yu. G. Talaeva, et al., in: Proceedings of an All-Union Conference on Water Hygiene and Health Protection of Reservoirs [in Russian], Moscow (1969), pp. 140-142.
12. C. E. Haley, R. A. Gunn, and J. M. Hughes, J. Infect. Dis., 141, 794 (1980).
13. H. Metz, Zbl. Bakt., 172, 255 (1980).
14. P. Rajaseran, P. R. Dutt, and K. A. Pisharoti, Ind. J. Med. Res., 66, 189 (1977).
15. "Waterborne infectious disease in Britain," J. Hyg. (London), 81, 139 (1978).