Considering data in the literature and the results of the present experiments on activation of phage RNA polymerase and synthesis of phage DNA and RNA by DMSO, it can be concluded that one mechanism of its action is by its activation of phage RNA polymerase in vivo. Since this enzyme is essential both for transcription of the gene coding DNA polymerase and for the initiation of DNA synthesis [3], it can be postulated that the stimulant action of DMSO on the synthesis of phage DNA is the result of activation of phage RNA polymerase. This is confirmed by the following facts: 1) maximal activation, constant in value, of reproduction of phage T-3 by DMSO was observed during the first 6 min of the latent period, i.e., the period sensitive to the action of the compound was between the 5th and 6th minutes, when transcription of the first genes of the "late region" – of DNase, DNA polymerase, etc. – by phage RNA polymerase begins [2]; 2) at the same time DMSO did not activate the reproduction of phages T-4 and λ which, unlike phages T-3 and T-7, utilize the intracellular RNA polymerase after modifying it.

The results showing that rimantidine selectively blocks phage RNA polymerase and the hypotheses expressed above regarding the possible mechanism of action of DMSO formed the basis for studies of the action of these compounds when used together. Experiments showed partial blocking of their mutually opposite action on the synthesis of phage macromolecules (Fig. 3) and the total abolition of the inhibitory action of rimantidine on the yield of infectious phage T-3.

The hypotheses regarding the action of rimantidine and DMSO expressed above can serve as the basis for further study of the mechanisms of action of these compounds on the structural and functional components of phages.

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NAG INFECTION IN TADPOLES OF Rana temporaria

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In a series of experiments 2250 tadpoles were infected with three strains of NAG vibrios. It can be concluded from the results of bacteriological and pathomorphological electron-microscopic and light-optical investigations that during the first 2 days the animals develop and recover from an acute infection, but the vibrios later persist for a long time in the body of the tadpoles and are excreted with the feces into the surrounding medium.

KEY WORDS: frog tadpoles; NAG infection; vibrios; mitoses of intestinal epithelium.

The problem of cholera and NAG infection cannot be solved without elucidation of the nature of interepidemic periods. It is particularly important to discover the factors which maintain the endemicity of the infection

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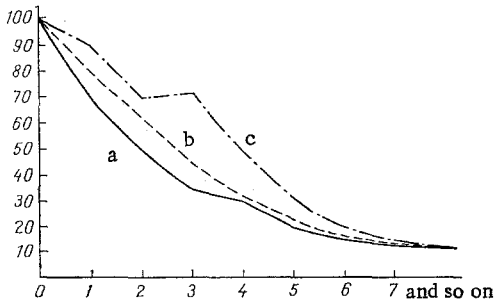

Fig. 1. Dynamics of positive cultures of NAG-vibrios from tadpoles. Ordinate, positive cultures of NAG vibrios (in %); abscissa, time after infection (in days). a) Group of animals infected with strain No. 75 of NAG vibrio; b) group infected with strain 318/g of NAG vibrios; c) group infected with strain A-167 of NAG vibrio.

in the foci and cause fresh outbreaks of the disease. There are reports in the literature that vibrios can be preserved for a long time, up to 2 years, in static water tanks, and can even survive the winter; there is also evidence of the role of hydrobionts as temporary carriers of vibrios. However, no investigator has noted whether the hydrobionts develop an illness after infection or whether the vibrios multiply in these organisms [2-4, 7-9].

The writers showed previously [5] that larvae of Rana temporaria are highly sensitive biological reagents for the exotoxin choleragen. These observations, in our view suggests that vibrios can produce an infectious disease in hydrobionics.

It was accordingly decided to undertake investigations to study the possibility that tadpoles may develop an NAG infection, to determine whether the vibrios multiply in them, and to establish the duration of their persistence in the body of the tadpole.

EXPERIMENTAL METHOD

Altogether 3000 tadpoles aged from 8 to 30 days were used; they were infected with the following strains of NAG vibrios: No. 75, 318/g, and A-167 (750 tadpoles for each strain). For this purpose, 250 tadpoles were kept for 2 h in water infected with one of the strains of vibrios ($7 \cdot 10^9$ bacterial cells to 100 ml water). The animals were then washed five times with dechlorinated water and transferred to glass crystallization tanks in 500 ml of aerated tap water. The experimental and control tadpoles were kept under identical conditions at a temperature of 20-24°C, washed daily with dechlorinated water, and fed with pulped fresh nettle. Bacteriological tests on homogenates of five tadpoles and homogenates of five intestines of the animals of all the experimental groups and of the water in which they lived were carried out 2 h after infection (the initial time), when the number of vibrios isolated was taken as 100%, and then daily until the end of the investigations for 30 days. The homogenates and their dilutions, as well as water from the experimental crystallization tanks, were seeded on dishes with TCBS, Endo's and Levine's media. The seedings were read after incubation for 18 h at 37°C.

The technique of the electron-microscopic investigations was described previously [1]. Tadpoles for histological investigation were fixed in Carnoy's mixture. A segment of small intestine was embedded in paraffin wax and transverse sections 5 μ in thickness were cut and stained with hematoxylin–eosin. Mitoses were counted in 10 sections in each case, i.e., on the average in 2000-3000 enterocytes.

EXPERIMENTAL RESULTS

During the first 3 days many colonies capable of being counted in dilutions were found on the dishes with TCBS medium. On the fourth to fifth day and later the vibrios in homogenates of the tadpoles tissues could not be estimated quantitatively, for there were few of them and they did not grow on TCBS medium. Vibrios were seeded for 30 days from water in which the tadpoles of the experimental groups were kept. The presence of vibrios in the medium in which the tadpoles lived indicated that they continued to excrete the vibrios into the medium, and for that reason after the fifth to sixth day the bacteriological tests for the presence of vibrios in

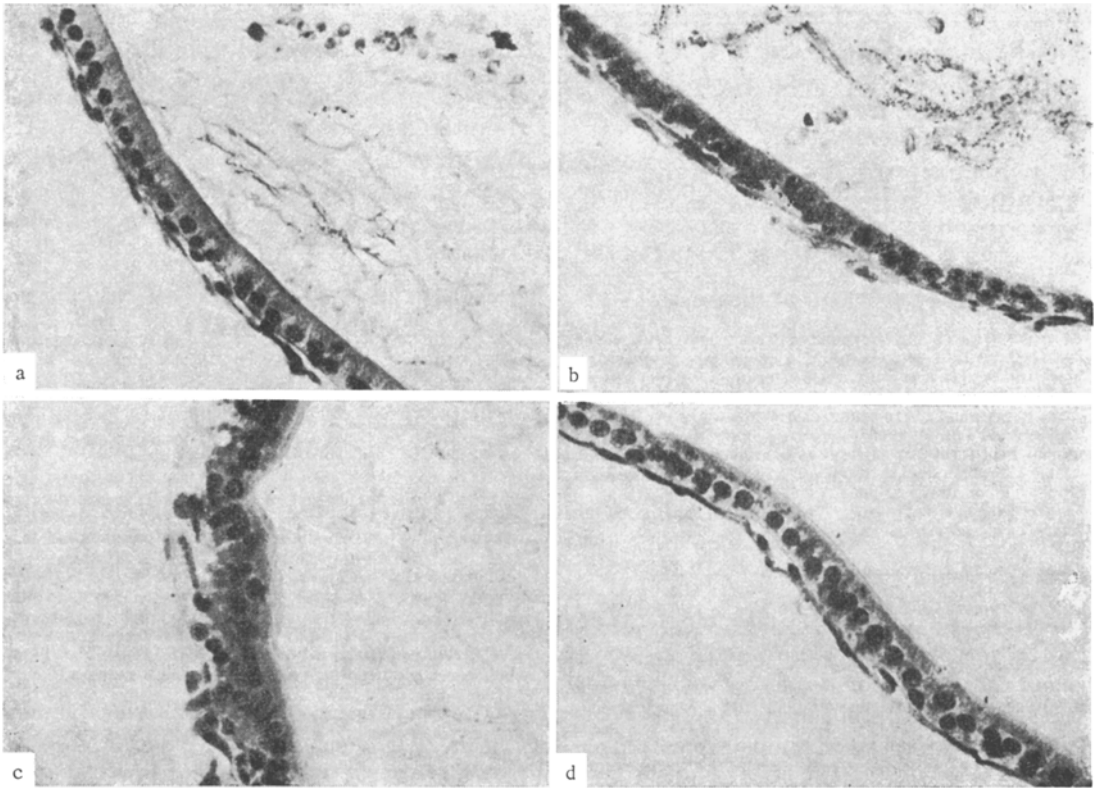

Fig. 2. Small intestine of tadpole: a) intact animals; b) 1 day after infection with NAG vibrios; c) 3 days after infection with a strain of NAG vibrio; d) 10 days after infection with a strain of NAG vibrio. Hematoxylin-eosin, 500 ×.

the tadpoles were qualitative only. Homogenate from the tissues of five tadpoles, its dilutions, and also the water in which the tadpoles lived were seeded on alkaline peptone water (enrichment medium) followed by reseeding on TCBS medium.

It will be clear from Fig. 1 that the number of vibrios seeded from tadpoles decreased daily, and on the fifth day after infection the presence of vibrios in the animals could be determined only qualitatively. The largest number of vibrios was seeded from tadpoles of the experimental group infected with strain A-167 of NAG vibrio. In these animals the number of vibrios on the second to third day became stabilized, but on the fifth to sixth day the presence of vibrios in the intestine of the animals could be determined only qualitatively. Second place as regards the number of positive cultures was occupied by the group of tadpoles infected with strain 318/g of NAG vibrio, followed in third place by animals infected with strain No. 75. It will be noted that on the second to third day the water in the experimental crystallization tanks (especially in the case of infection with strain 318-g) was yellow in color and contained no solid particles of feces. In the crystallization tanks containing intact tadpoles the water was transparent and contained solid particles of feces. No difference was found in the behavior of the animals of the experimental and control groups. At autopsy on tadpoles of the experimental groups 18-72 h after the time of infection, the intestine was swollen, easily torn with forceps, and had gelantinous contents. From the fourth day and until the end of the experiments no visible changes were observed in the intestine.

It accordingly appeared interesting to study the dynamics of development of the pathological changes and the repair processes in the small intestine of tadpoles infected with the vibrios.

On electron-microscopic investigation of epithelial cells of the small intestine 2-4 h after exposure to NAG toxin changes were discovered in the apical portion of the cells and were focal in character. They took the form of deformation of the microvilli, increased vesicle formation, enlargement of the diameter and deformation of the mitochondria: Condensation and homogenization of their matrix were found at the same time.

After 20-24 h the changes described above in the epithelial cells were joined by edema of the basal part of the cells and by the appearance of lysosomes in larger numbers than 2 h after the beginning of action of the toxin. The intercellular spaces of the epithelial layer were widened, especially in the basal part.

The endothelial cells in the blood capillaries of the submucous layer were edematous, the noncellular component of the basal layer was thin and friable, and the pericapillary zone also was edematous.

The results of the electron-microscopic investigation thus show that during the first 2 h after the beginning of action of the enterotoxin of the NAG vibrios on the epithelial cells of the intestine its focal cytotoxic effect was visible, and by 20-24 h after the beginning of exposure increased outflow of fluid from the capillaries was added to these changes. Migration of fluid from the pericapillary zone into the basal part of the epithelial cells could also be deduced from the widening of the intercellular spaces in the basal part of the epithelial layer of the intestine and the edema of the epithelial cells in this zone.

Light-optical investigations of the sections showed that 24 h after infection the height of the epithelium and the thickness of its brush border were reduced in the small intestine of tadpoles of R. temporaria (Fig. 2a, b). Around the nucleus there was a thin rim of cytoplasm. Sometimes the nuclei were irregular in shape. Hardly any mitoses were present in the enterocytes. On the third day the state of the enterocytes was close to normal. The nuclei were arranged in several rows and were swollen; most of them were in the early phases of cell division. The mitotic index (MI) was sharply increased: Whereas in intact tadpoles at the same stage of development it was 18.76 $^0/_{00}$, in the experimental series MI was 132 $^0/_{00}$, i.e., proliferation was increased sevenfold (Fig. 2c). On the 10th day the microscopic structure of the enterocytes was normal (Fig. 2d). MI in the control group was 19.60 $^0/_{00}$ and in the experimental group 28.33$^0/_{00}$. The prolonged increase in the intensity of proliferation was evidently due to the fact that the tadpoles remained carriers of vibrios for a long time.

The results of the clinical, bacteriological, electron-microscopic, and light-optical investigations show that the tadpoles developed an acute infectious disease during the first 2 days, and thereafter the vibrios persisted in the body of the tadpoles for up to 30 days and were excreted with the feces into the surrounding medium. This opens up fresh aspects in the study of the ecological interrelations between vibrios and the surrounding medium and it indicates that hydrobionts may play a role in the maintenance of the endemicity of infections caused by vibrios.

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