EFFECT OF THE COMBINED ACTION OF RIMANTIDINE AND DIMETHYLSULFOXIDE ON PHAGE REPRODUCTION

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The effect of dimethylsulfoxide (DMSO) and rimantidine on the reproduction of phages T-3, T-4, T-7, and λ were studied singly and in combination. Maximal activation of reproduction of phages T-3 and T-7 by DMSO was observed during the first 6 min of the latent period. Meanwhile this compound had no action on reproduction of phages T-4 and λ, a result attributable to the use of intracellular RNA polymerase by these phages. DMSO was shown to stimulate synthesis of the DNA of phage T-3, evidently through its action on phage RNA polymerase. During the combined action of these compounds, partial blocking of their mutually opposite action on the synthesis of phage macromolecules and total abolition of the inhibitory action of remantidine on the yield of infectious phage T-3 were observed.

KEY WORDS: dimethylsulfoxide; rimantidine; phage; RNA-polymerase.

In recent years dimethylsulfoxide (DMSO) has attracted the attention of research workers. This compound has been shown, on the one hand, to increase the infectivity of the RNA of poliomyelitis and Mengo viruses, and on the other hand, to accelerate penetration of influenza virus into the cell. In addition, DMSO increases interferon production in mice infected with Sindbis and Chalovo viruses [5]. In subbacteriostatic concentrations it increases the penetration of dyes, antibiotics, and other biologically active compounds into bacterial cells, and if its concentration is increased to 25-30%, it produces lysis of the cell walls. This compound is interesting also from the standpoint of its action on certain enzyme systems. For instance, Strätling et al. [6] showed that DMSO in vitro stimulates the synthesis of DNA of phage T-7 and activates the function of phage RNA polymerase.

The object of this investigation was to study the effect of DMSO on reproduction of phages T-3, T-4, T-7, and λ and to examine the effectiveness of its use in combination with rimantidine, which has the property of inhibiting phage RNA polymerase.

EXPERIMENTAL METHOD

Experiments were carried out with the following strains of Escherichia coli: E. coli B, E. coli BB, E. coli K-12 (λ), E. coli K-12 CSH-2 (R-4), pro⁻ met⁻ (λ), and E. coli K-12 SF-14 (try⁻, S^r), and with phages T-3, T-4, T-7, and λ. DMSO and rimantidine were dissolved in medium M-9, which was used as the nutrient medium for growth of the bacteria. The action of the compounds on phage reproduction was studied by the usual methods [1]. To study the action of these compounds on a phage model, investigations with radioactive precursors were carried out by the method described previously [2].

EXPERIMENTAL RESULTS

As a first step the concentrations of DMSO close to the maximal tolerable levels were determined. The compound, in a concentration of below 3%, had no bacteriostatic action on any strain of E. coli used during incubation for 1 to 24 h, in agreement with data obtained earlier.

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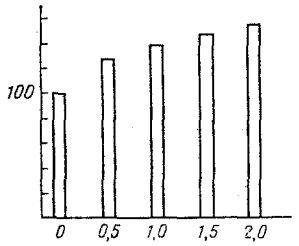

Fig. 1

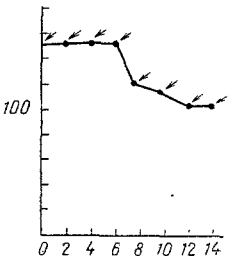

Fig. 2

Fig. 1. Stimulating action of various concentrations of DMSO on production of infectious phages T-3 and T-7. Abscissa, concentration of DMSO (in %); ordinate, here and in Fig. 2, production of infectious phage (in % of control).

Fig. 2. Action of 2% DMSO on yield of infectious phage as a function of time of addition of compound. Arrows indicate time of addition of DMSO. Abscissa, time (in min).

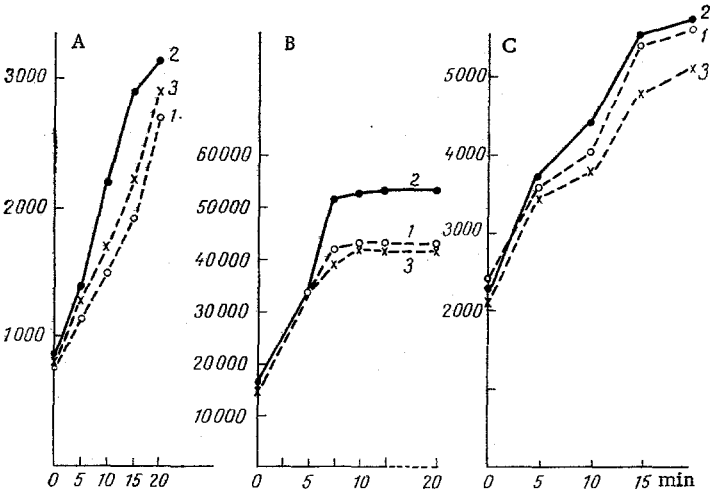

Fig. 3. Effect of DMSO (2%) alone and in conjunction with rimantidine (100 μg/ml) on synthesis of proteins (C) of phage T-3. DNA (A), RNA (B). 1) Infected bacteria (control); 2) 2% DMSO; 3) 2% DMSO + rimantidine (100 μg/ml). Abscissa, time (in min); ordinate, intensity of synthesis (in counts/min).

In the course of this period of incubation, 2% DMSO had no significant effect on the metabolism of the bacterial cells, as shown by incorporation of precursors into bacterial DNA, RNA, and proteins. Meanwhile the compound had no phagocytic action on phages T-3, T-4, T-7, and λ.

The next step was to study the effect of various nonbacteriostatic concentrations of DMSO on the reproduction of these phages. Its action on phages T-4 and λ was negligible, but the degree of activation of reproduction of phages T-3 and T-7 depended on the concentration of the compound (Fig. 1).

The next experiments were carried out with 2% DMSO. The object of this series of experiments was to study how the action of DMSO on reproduction of phages T-3 and T-7 depends on the time of its addition to the phage–bacterium system. The results (Fig. 2) showed that the effectiveness of the compound was greatest during the first minutes of the latent period of development of the phage.

The mechanism of action of DMSO on reproduction of phage T-3 was judged from the incorporation of thymidine-^{3}H and uridine-^{3}H into phage DNA and RNA and of amino acids-^{14}C into proteins. It will be clear from Fig. 3 that in the presence of 2% DMSO the synthesis of phage DNA was increased by 50%, of RNA by 25%, and of protein by 10%.

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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