# MUTATIONS OF THE ht-SIGN IN BACTERIOPHAGE T-2, INDUCED BY THE COMBINED ACTION OF CHEMICAL MUTAGENS

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The purpose of this work was to study the combined mutation action of hydrazine and sarcolysin on the extracellular phage, T-2. The indicated compounds were investigated as inducers of ht-mutations, i.e., as factors altering the sign of the phage's action range. Mutation of the phage T-2h$^+$ to T-2h involves the capacity of the mutants to lyse not only the usual bacterial host (E. coli B), but also the culture of E. coli B/2, resistant to the action of the original phage.

The nutagens used by us are characterized by the capacity to interact only with certain nitrogenous bases of DNA. Hydrazine ($N_2H_4OH$) leads to destruction of the pyrimidine ring of thymine [3,5], while sarcolysin interacts with guanine by alkylating nitrogen in the 7th position [4]. Thus, a system of the two indicated mutagens can interact with both pairs of nitrogenous bases in the DNA of the T-2 phage. According to Freeze [2], the subsequent replication of this DNA can lead to simple or complex changes in the nitrogenous bases within its nucleotides.

## EXPERIMENTAL METHOD

We treated 0.2 ml of a phage T-2 concentrate, containing $10^{12}$ particles per ml, with 9.8 ml of a 0.1 M aqueous solution of hydrazine, at pH 8.2. The degree of inactivation of the phage was determined by seeding a test sample according to the method of Gratsia [1].

A concentrate of the phage T-2 (0.2 ml) was treated with 9.8 ml of a 0.01 M aqueous solution of sarcolysin, at pH 7.0. The degree of inactivation was determined in the same fashion as with the action of hydrazine.

The combined treatment of the T-2 phage with the indicated mutagens was accomplished in this sequence. The concentrate of T-2 phage with hydrazine was incubated for 24 h, following which it was washed off once, in a rapid centrifuge (18,000 rpm), and resuspended in 1 ml of the saline medium M-9. After this, 0.2 ml of this suspension was treated with 9.8 ml of a 0.01 M solution of sarcolysin, for a period of 90 min.

In the second variation of the experiments, the sequence for treating the phage was reversed. As a control, we used suspensions of the phage that were twice treated with hydrazine (24 h for each treatment) or sarcolysin (90 min for each treatment).

The number of ht-mutants was determined in the following manner: 0.5 ml of treated phage was diluted with M-9 medium by 100 times, and 1 ml of this dilution was incubated for 5 min at 37° with 3 ml of a culture of E. coli B (preadsorption); then, this mixture was seeded out on a "lawn" of E. coli B/2 culture, resistant to the original phage. The dishes were incubated at 37° for 18 h, after which we counted the number of negative ht-colonies.

The mutagenic activity of each of the mutagens and their combination was evaluated by determining the ratio m/v, where $\underline{m}$ is equal to the number of induced mutants, and $\underline{v}$, the amount of activity of the phages.

## EXPERIMENTAL RESULTS

From the table, in which the results of the experiments are summated, it can be seen that separate treatment of the phage T-2 with hydrazine or sarcolysin led to low induction of ht-mutations. In this case, we noted that the index m/v was similar for the two mutagens.

Induction of ht-Mutations in Phage T-2, Treated with Sarcolysin, Hydrazine, and their Combination (Average of 3 Experiments)

| Mutagen | $\frac{m_0}{v_0}$ | $\frac{m_t}{v_t}$ |
|---|---|---|
| Hydrazine | $3{,}3 \cdot 10^{-7}$ | $2{,}6 \cdot 10^{-6}$ |
| Sarcolysin | $5{,}3 \cdot 10^{-7}$ | $4{,}3 \cdot 10^{-6}$ |
| Hydrazine + Sarcolysin | $8{,}5 \cdot 10^{-6}$ | $5 \cdot 10^{-2}$ |
| Hydrazine + Hydrazine | $10 \cdot 10^{-6}$ | $7 \cdot 10^{-4}$ |
| Sarcolysin + Sarcolysin | $9 \cdot 10^{-6}$ | $2 \cdot 10^{-4}$ |

Note. The amount of active phages after treatment with hydrazine or sarcolysin was taken as 100%.

Double treatment with sarcolysin did not cause an increase in the value of m/v, but double treatment with hydrazine intensified the induction of mutations. Combination of both mutagens, regardless of the sequence of the treatments, caused an increase in the rate of mutagenesis.

The mechanism of the described effect, i.e., marked increase in the induction of mutations subsequent to the combined action of two weak mutagens, is still not clear. It can only be postulated that the damage to the DNA caused by the combination of effects, i.e., as a result of damage to both pairs of bases, creates large possibilities, during the subsequent replication, for changes in the sequence of the DNA nucleotides within the phage, which is manifested by an intensification of the mutation effect.


## SUMMARY

Combined treatment of the extracellular bacteriophage T-2 with two weak mutagens (sarcolysin and hydrazine), each of which gives a low induction of ht-mutants, led to marked intensification of the mutation effect. It is postulated that this phenomenon may be explained on the basis of the Freese hypothesis, i.e., that it is a result of extensive possibilities for changes in the DNA nucleotide sequences within the T-2 phage.


## LITERATURE CITED


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