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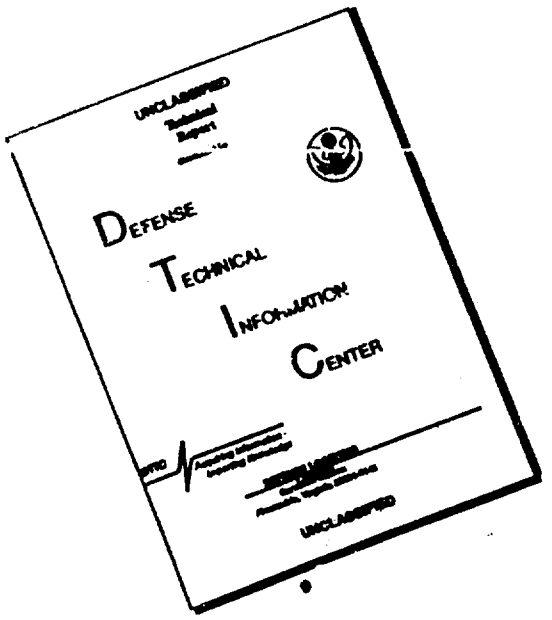

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POSSIBILITY OF DEPRESSING THE TOBACCO MOSAIC TITER VIRUS WITHOUT DISTURBING NUCLEIC ACID SYNTHESIS IN THE CELL

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All modern chemotherapeutic remedies against bacterial pathogenic agents are ineffective against viral diseases. This is explained by the fact that the nature of viruses is basically different from the nature of microorganisms. No matter how small a bacterial cell is, it has its own metabolism which is different to some degree from the metabolism of the host cell. Taking advantage of these differences, it was possible to develop preparations which were toxic to the bacterial cell and harmless to the human organism.

Thus, the application of sulfonamide became possible due to the ability of bacterial cells to synthesize folic acid and the loss of this ability by animal cells. By inhibiting one of the enzymes participating in the synthesis of folic acid, sulfonamides disturb its synthesis, which eventually results in the death of the bacteria. Animal cells satisfy their need in folic acid at the expense of food assimilation. Therefore, causing the death of bacterial cells, sulfonamides are nontoxic to man. There are also preparations in medical practice whose application is based on the different degree of permeability of cellular membranes of bacterial and animal cells.

Streptomycin, passing through the membranes of the bacteria, causes their death. Human cellular membrane is impermeable to streptomycin and, therefore, it is nontoxic to man.

Viruses, unlike microorganisms, have neither a membrane nor their own metabolism; therefore, it is not clear whether it will be possible to apply to viral disease the same principles on which chemotherapy of bacterial diseases is based.

A different approach to the treatment of viral and bacterial diseases is also based on the fact that bacteria and viruses do not multiply in the same way.

As soon as a bacterial cell is killed and deprived of its ability to multiply, the number of the daughter cells in the population decreases sharply and, consequently, their pathogenic effect on the organism weakens sharply.

Viruses do not multiply in the true sense of the word. More likely they do not have mother or daughter cells. Having entered a cell, virus decomposes. It is impossible to detect it during the early stages of infection either by the chemical or serological methods. During this period the cell metabolism changes in such a way that the cell begins to synthesize viral nucleoprotein; therefore, no matter how much we inhibit the infectious nature of the developed viral particles, the infected cell will probably continue synthesizing new particles. Consequently, in order to stop the "multiplication" of a virus, it is necessary to be able to change the metabolism of the cell in such a way that it would lose the ability to synthesize viral nucleoprotein.

Thus, the difference in the way of multiplication of bacteria and viruses results in the fact that the "target" of the action of the curative preparation is the pathogenic agent itself in one case, and the metabolism of the host cell in the other.

To change the metabolism of the infected cell in the right direction is still a problem without a solution. This is why medical science still does not have preparations depressing the "multiplication" of viruses.

A considerable number of substances depressing the accumulation of viruses have been found experimentally, but all of them are toxic to the cell itself.

It is not surprising that doubts have been expressed in literature regarding the possibility of depressing the "multiplication" of viruses without upsetting the synthesis of cellular nucleoproteins. "In principle, the synthesis mechanism

of a virus in a cell is the same as the mechanism of building up the cell's own substance... Therefore, it is difficult to imagine substances that are capable of distinguishing these two identical mechanisms which differ from one another only in their final products. Therefore, the solution of the problem of chemotherapy of viral diseases gives very little hope"(Ivanovich, 1963). It is true that it is difficult to search for substances which would differentiate between the differences in the synthesis of viral and cellular nucleoproteins, not even knowing what differences there are in the synthesis of these two nucleoproteins. Therefore, we have conducted studies for determining the changes which are caused by the virus in the synthesis of nucleic acids.

In 1962, we (Nikiforova, 1962) and Reddi, in 1963, showed that tobacco mosaic virus (TMV) causes an increased decomposition of cellular nucleic acids. However, the index of the degree of decomposition of cellular nucleic acids, by itself, is not a qualitative distinction of a sick cell from a healthy one, since an increased decomposition of the nucleic acids in the cell can be caused by a number of other factors. For the first time we (Nikiforova, 1964) discovered a qualitative change in the nucleic metabolism of tobacco caused by the multiplication of the TMV. This change was expressed by the fact that the amount of nucleic acids of healthy and infected tobaccos changed in different ways when the same purine and pyrimidine bases were introduced into a cell.

Thus, adenine increased the amount of nucleic acids in healthy tobaccos but lowered the TMV titer and the total (cellular + viral) nucleic acids in the infected tobaccos. Guanin, on the contrary, sharply increased the TMV titer and the total nucleic acid in the infected tobaccos and had almost no effect on the content of nucleic acids in healthy tobaccos.

Inasmuch as the changes of the virus titer and the total nucleic acid in the infected cells were well-defined (i.e. the content of total nucleic acid increased with the increase in the TMV titer, and vice versa), it remained unexplained whether the entire nucleic metabolism changes in the infected cell or only some isolated section of it.

The fact that plants infected with a virus do not die but continue to grow and even produce fruit testifies to the presence of the synthesis of cellular nucleic acids in the infected cells along with the synthesis of viral nucleoprotein. Therefore, it was necessary to determine how close the connection is between the syntheses of the cellular and viral

nucleic acids in the same infected cell. Is it possible to create such conditions in a cell when the synthesis of viral nucleic acid will be depressed without disturbing the synthesis of cellular nucleic acid? Or are both syntheses so closely connected that any effect depressing the synthesis of viral nucleic acid will unavoidably depress the synthesis of cellular nucleic acid?

An answer to this question was obtained by us from the experiments on feeding infected tobaccos with the products of fermentative decomposition of nucleic acids. The experiment was conducted on growing tobaccos. On the day of the experiment, average samples were taken from the experimental tobaccos and separate samples from the control tobaccos in order to determine the content of nucleic acids before infection. After the samples had been taken, the tobaccos were infected with the TMV. Starting on the third day after the infection, a hydrolyzate of ribonucleic acid (RNA) was introduced daily into the experimental tobaccos by brushing a hydrolyzate solution on the surface of the leaf.

An average sample was taken 5, 7, 9 and 12 days after the infection in order to determine the TMV titer and the content of nucleic acids in the experimental and control tobaccos.

The method of the analysis for the determination of the amount of nucleic acids was described by us earlier (Nikiforova 1962).

The TMV titer was determined by counting the necroses on the leaves of N. glutinosa. At the same time three variants of the experiment were set up under the same conditions on tobaccos of the same age: in the I variant, the infected tobaccos received a hydrolyzate of the yeast RNA (a commercial preparation); in the II variant -- an RNA hydrolyzate from TMV; in the III variant -- an RNA hydrolyzate from healthy plants.

In order to obtain nucleic acid, nucleoproteins were extracted from healthy tobacco leaves, first 0.14M NaCl, then 1M NaCl and 0.2% NaOH. The nucleoproteins precipitated at the isoelectric point were combined and subjected to dialysis against water. The RNA hydrolyzate from TMV was obtained from a purified TMV preparation prepared by the Nikiforova's method (Nikiforova, 1959). The deproteinization of the virus and the nucleoproteins isolated from healthy leaves was achieved by a thermal treatment. The solutions were brought to pH 6.5, a solution of ribonuclease was added and the flasks were placed in a thermostatically-controlled chamber at 38° for 48 hours. After being exposed for this period, the solutions were boiled in order to denaturize the ribonuclease. The precipitate was filtered through a paper filter.

The hydrolyzates were condensed by evaporation to a required concentration (500 γ/ml).

Our study has shown that when RNA hydrolyzates are introduced on the third day after the infection, the TMV titer is depressed. When a hydrolyzate of the yeast RNA and a RNA hydrolyzate from healthy leaves are introduced, the content of nucleic acids becomes higher. An RNA hydrolyzate from TMV causes a decrease in the content of nucleic acids (Figure 1).

We determined the total nucleic acid; the fact that its amount increases with the reduction of the titer gives us reason to believe that this increase was caused by the increase in the content of the cellular nucleic acid. By the changes in the virus titer it is possible, indirectly, to make some conclusions in regard to the changes in the content of the viral nucleic acid. According to the obtained data, in an infected cell it is possible to depress the synthesis of viral nucleic acid and to increase the content of cellular nucliec acid.

This disunity is apparently achieved because the synthesis of nucleic acids depends on the ratios of the RNA decomposition products in the introduced hydrolyzate.

Inasmuch as the cellular and viral nucleic acids differ in the composition of their bases, their synthesis will respond differently to the same introduced hydrolyzate. And, on the contrary, the synthesis of the same nucleic acid will change differently if the composition of the introduced RNA hydrolyzates is different.

The significance of the composition of the introduced hydrolyzate for the accumulation of the virus was particularly clearly shown in the experiments when the RNA hydrolyzates were introduced into the plants simultaneously with the virus (the virus preparation was diluted with the hydrolyzate instead of water). In this case the hydrolyzate of the yeast RNA lowered the titer of the TMV by 3 times, while the RNA hydrolyzate from TMV increased the titer by 3 times (see table).

The synthesis of the cellular nucleic acids of healthy tobaccos is just as sensitive to the composition of the introduced hydrolyzates.

This was shown by us in the experiments with healthy tobaccos. The experiment was conducted in the same way as

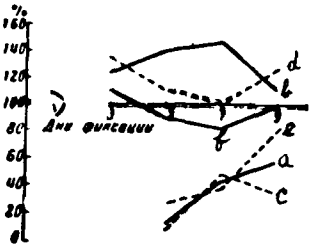

Figure 1. Effect of RNA decomposition products of various origins on the content of nucleic acids and TMV titers in infected tobaccos (in % of control for each day of experiment)
a -- TMV titer under the effect of nucleic acid hydrolyzate from healthy leaves;
b -- nucleic acid content under the effect of nucleic acid hydrolyzate from healthy leaves;
c -- TMV titer under the effect of a hydrolyzate of a commercial preparation of yeast nucleic acid;
d -- Nucleic acid content under the effect of a hydrolyzate of a commercial preparation of yeast nucleic acid;
e -- TMV titer under the effect of RNA hydrolyzate from TMV;
f -- Nucleic acid content under the effect of RNA hydrolyzate from TMV.
Legend: 1) Fixation days.

that with infected tobaccos. The content of nucleic acids of healthy tobaccos of all three variants was determined. The first sample was taken before the introduction of the hydrolyzate, and then 3, 5, 7, 9 and 12 days after the commencement of the experiment. Healthy tobaccos into which no hydrolyzates were introduced served as control.

The conducted analyses have shown that hydrolyzates of the yeast RNA and viral RNA lowered the synthesis of cellular nucleic acids. RNA hydrolyzate separated from healthy tobaccos increased the content of the cellular nucleic acid (figure 2).

Figure 2. Effect of the decomposition products of RNA of various origins on the content of nucleic acids in the healthy tissues (nucleic acid content on the first day of the experiment is considered to be 100%).
a -- control; b -- hydrolyzate of a commercial preparation of yeast nucleic acid;
c -- hydrolyzate of healthy nucleic acid;
d -- hydrolyzate of nucleic acid from TMV.
Legend: 1) Fixation days.

Dependence of TMV Titer on the Origin of RNA

Origin of RNA	Number of necroses		% in relation to control
	Experiment	Control	
Hydrolyzate of yeast RNA	122	353	34
Hydrolyzate of RNA from TMV	249	923	370
Hydrolyzate of RNA from healthy tobaccos	347	285	121

Consequently, by a definite composition of the introduced RNA hydrolyzates it is possible to regulate the direction of the synthesis of nucleic acids either upward or downward.

Thus, it is possible to select such a composition of a RNA hydrolyzate that will permit to reduce the synthesis of

the viral nucleic acid to a minimum and at the same time to increase the content of the cellular nucleic acid in the infected cell.

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