

ON THE POSSIBLE ROLE OF RIBONUCLEIC ACID IN THE MECHANISM
OF ANTITUBERCULOSIS RESISTANCE.

REPORT II. RIBONUCLEIC ACID AND THE RIBONUCLEASE ACTIVITY
IN THE ORGANS OF BCG VACCINATED AND TUBERCULOSIS
INFECTED MICE

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In the previous study [1] we found that immunization against tuberculosis brings about an increase in the level of ribonucleic acid (RNA) in organs of mice and guinea pigs, and infection with a virulent strain of tubercle mycobacteria increases the amount of RNA in lungs of mice but has no effect on the content of RNA in lungs of guinea pigs.

The present work was concerned with elucidation of the mechanism of RNA metabolism in mice during the development of artificial immunity as a result of injection of BCG vaccine and of infection with mycobacteria of tuberculosis. Since ribonuclease is one of the most important enzymes involved in RNA metabolism, we investigated the connection between the activity of this enzyme and the content of RNA in organs of animals under the above indicated experimental conditions.

EXPERIMENTAL METHODS

In the first series of experiments we studied the effect of single and double immunization on RNA content and on the activity of ribonuclease in lungs and liver of white mice.

Mice were immunized subcutaneously with 1 mg of BCG vaccine; the second injection was administered 2½ months after the first. The single injection of the first group of mice was carried out at the same time as the immunization of the second group with the repeated dose of vaccine. Six mice from each experimental group and six control, not immunized animals were sacrificed simultaneously on the 4th, 11th, 20th, 38th, 67th and 98th days after the last immunization.

In the second experimental series we investigated the content of RNA and the activity of ribonuclease in mice infected with virulent strain of *M. tuberculosis* No. 109. The dose and the method of infection were the same as used in the previous study [1]. Six experimental and 6 control mice were sacrificed on the 4th, 11th, 20th, 35th, 38th, 65th and 90th days after infection.

Sodium ribonuclease preparation, obtained by us from yeasts according to the method described by Holden and Pirie [2], was used as a substrate for determination of activity of ribonuclease.

One ml of acetate buffer at pH 5.6 and 0.5 ml of 1% of liver and lung homogenate were added to 0.5 ml of sodium ribonuclease solution (containing about 200 mg of phosphorus in 1 ml). Two ml of 0.25% of uranyl acetate solution, prepared in 10% solution of trichloroacetic acid, were added to samples incubated for 1 hour in a water bath at 37°C. Uranyl acetate solution was added prior to incubation to control samples. After addition of uranyl acetate the samples were left for half an hour at 25°C and were then centrifuged. At the same time samples were set up for testing spontaneous decomposition of RNA under conditions of hour-long incubation (physiologic solution was added instead of the homogenate). Acid soluble phosphate was determined using Fisk-Sabborough procedure.

The activity of ribonuclease was expressed in milligrams of acid soluble phosphate, removed from RNA in samples during hour-long incubation (deducted from phosphorus spontaneously removed from RNA under experimental conditions.*

* Increase in acid soluble phosphorus in samples containing physiological solution in place of RNA (a sample for separation of acid insoluble compounds of the homogenate) was insignificant, therefore this control was not prepared in subsequent experiments.

EXPERIMENTAL RESULTS

The results of the study are shown in Figs. 1 and 2. In comparing the effectiveness of single and double immunization on the level of RNA (Figs. 1 and 2, c, d) and ribonuclease activity (Figs. 1 and 2, a, b) in lungs and liver

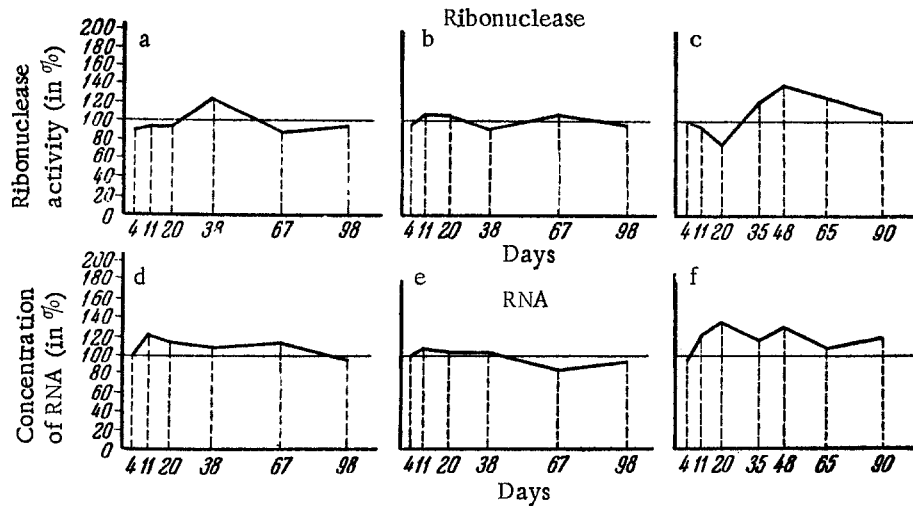


Fig. 1. Activity of ribonuclease and concentration of RNA in lungs. Ribonuclease: a) singly immunized mice; b) doubly immunized mice; c) infected mice. RNA: d) singly immunized mice; e) doubly immunized mice; f) infected mice. Curves are drawn in accordance with the following consideration: conditions in experimental animals were related to similar conditions in control animals, sacrificed simultaneously, and expressed as per cent; control is always considered to be 100%.

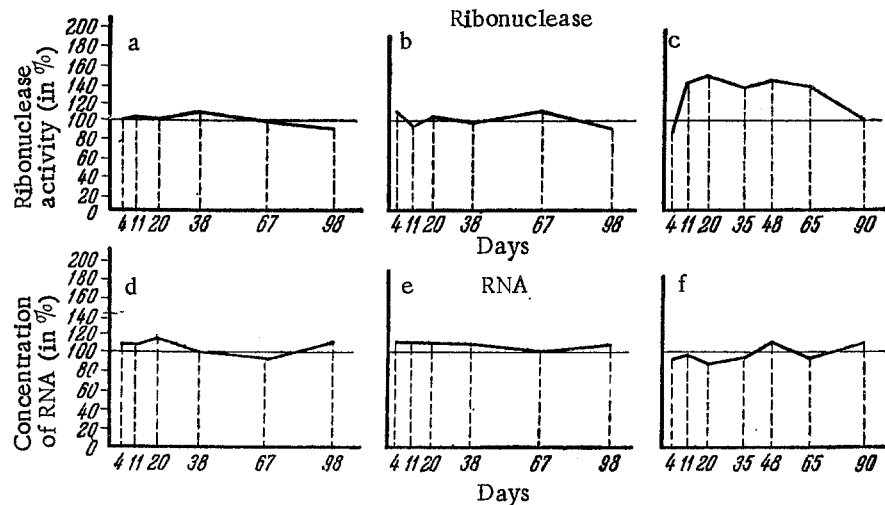


Fig. 2. Activity of ribonuclease and concentration of RNA in liver. Ribonuclease: a) in singly immunized mice; b) in doubly immunized mice; c) in infected mice. RNA: d) in singly immunized mice; e) in doubly immunized mice; f) in infected mice. The basis for preparation of the curves is similar to that in Fig. 1.

of mice, it should be noted that, if in accordance with our previous experiments [1] single immunization brought about a significant increase in RNA content in organs of mice (by 8-19% in the lungs and 7-18% in the liver) and phasic changes in ribonuclease activity in the lungs (increase in enzyme activity by 21% on the 38th day after immunization and decrease by 13% on the 48th day), then in organs of doubly immunized mice both indicators (RNA content and ribonuclease activity) varied in the range of control figures essentially for the duration of the entire experiment.

One of the possible explanations of this fact may lie in the supposition that repeated introduction of an attenuated strain of BCG acts on the organism already adapted to vaccination with BCG as a result of the initial immunization.

RNA of infected mouse lungs increased by 20% on the 11th day and remained at this level (16-33% above the control) for the duration of the experiment; on the other hand, concentration of liver RNA was considerably lower than that of the control (by 6-16%), returning to normal only on the 65th day after infection.

The changes in ribonuclease activity in lungs of infected mice, as well as in lungs of singly immunized mice, were phasic in nature: the enzyme activity decreased by 24% on the 20th day, then it began to rise, reaching 36% on the 65 day. By about the 90th day the ribonuclease activity had returned to its initial level. In the liver of infected mice enzyme activity was 20% lower than that of the control on the 4th day after infection. It rose sharply on the 11th day (by 35% above the control) and remained at this level for 2 months, returning to normal only on the 90th day after infection.

Comparing RNA concentration curves and curves for enzyme activity in lungs of infected and singly immunized mice, we found a similarity in the nature of those changes which are affected by immunization and infection.

The mechanisms, found in studying the lungs of immunized mice (increase in RNA level, phase variations in ribonuclease activity), are shown even clearer in lungs of infected animals. In this manner, the hypothesis expressed by us in the previous publication [1] that identical biochemical mechanisms constitute the basis of natural resistance and artificial immunization to tuberculosis, received additional confirmation.

As it has been indicated above, the present investigation was undertaken with the purpose of showing a relationship between alteration in ribonuclease activity and changes in RNA concentrations in the organs of animals infected with tubercle bacilli and immunized with BCG. In comparing curves a and b and c and f in Fig. 1, regardless of the absence of qualitative parallelism and exact agreement of dates, there appears an inverse relationship between the changes in these factors: decrease in ribonuclease activity, as a rule, is accompanied by increase in RNA concentration and, conversely, increase in ribonuclease activity is followed by decrease in RNA concentration. Apparently, ribonuclease is involved in these alterations in RNA level which take place in mouse lungs under the influence of immunization and infection with tuberculosis.

SUMMARY

In studying the RNA content and the ribonuclease activity in mice infected with tuberculosis and following a single and double BCG vaccination the following was revealed. Single immunization causes a rise of the ribonucleic acid content in the organs of mice, and phasic changes in the ribonuclease activity in mice lungs; after a double immunization these indices showed almost no difference from control. The curves of RNA level and of the ribonuclease activity in the lungs of the animals immunized once and of the infected ones were of the same character; however, the regularities noted in the immunized animals were even more pronounced in the infected animals. And, in reverse, relationship was noted between the changes occurring in the ribonuclease activity and in the ribonucleic acid level in the lungs of the BCG immunized animals and of those infected with bacillus tuberculosis.

LITERATURE CITED

1. E. B. Lishnevskaya, Byull. Éksper. Biol. i Med. No. 9 (1961), p. 63.
2. M. Holden and N. W. Pirie, Biochem. J. Vol. 60 (1955), p. 46.

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