

THE EFFECT OF THE SERUM OF IMMUNIZED GUINEA PIGS
ON THE MULTIPLICATION AND CATALASE ACTIVITY
OF *Mycobacterium tuberculosis*

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The serum and blood of rats—animals highly susceptible to tuberculosis—are known to possess the property of inhibiting growth of *Mycobacterium tuberculosis* [7, 12]. A similar property is possessed by the serum of rabbits sensitive to tuberculosis [14, 17], especially after immunization with BCG [10, 11, 14, 16, 20]. The possibility of a bacteriostatic action of serum from patients with tuberculosis on *M. tuberculosis* has been mentioned [13, 18], and some workers [9] consider that the active factor in the serum is its carbohydrate-lipid complex, whereas others [21] ascribe this role to ascorbic acid derivatives, lactic and fatty acids. One group of writers explains the bacteriostatic properties of the serum by the action of its γ -globulin fraction [22], a lysozyme-like enzyme [16, 17, 20], or its properdin system [1].

Attempts have been made to discover the biochemical basis of the effect of animal sera on *M. tuberculosis*. It has been found [3], for instance, that the serum of artificially immunized guinea pigs and of albino mice naturally resistant to tuberculosis depresses the oxidative assimilation of phosphorus by *M. tuberculosis* and stimulates the bacterial ATPase [4]. Some workers [19] consider that one stage in the bactericidal action of rat serum on *M. tuberculosis* is dephosphorylation of bacterial polysaccharides.

In the present research we have investigated the effect of the serum of immunized guinea pigs on the growth and catalase activity of *M. tuberculosis*. High catalase activity in *M. tuberculosis* is an indication of the biological efficiency and viability of the strain [5, 6]. Interest in the study of this enzyme has grown in particular since reports have appeared that it may be related to the virulence of *M. tuberculosis* [15].

EXPERIMENTAL METHOD

We used serum from healthy and immunized guinea pigs (1 mg dry BCG vaccine in 0.2 ml of physiological saline intradermally). Experiments were conducted with a bovine strain of *M. tuberculosis* (No. 23 of the Leningrad Institute of Tuberculosis). Multiplication of *M. tuberculosis* was studied in Soton's medium containing $\frac{1}{4}$ of its volume of serum of immunized guinea pigs. The catalase activity of the *M. tuberculosis* was estimated from the consumption of hydrogen peroxide in a medium containing a bacterial suspension exposed to the action of the serum of immunized guinea pigs. One series of experiments to study the catalase activity of the microorganisms was carried out in the presence of serum previously heated to 56° for 30 min. This was necessary to determine the degree of thermostability of the factor under investigation. Controls to all the experiments consisted of untreated mycobacteria and microorganisms incubated in the presence of the serum of normal guinea pigs.

EXPERIMENTAL RESULTS

We found that the serum of healthy guinea pigs had no effect on the growth of *M. tuberculosis* (Fig. 1; IIb, IIb, IVb). Immunization of the animals conferred bacteriostatic properties on their sera, detectable on the 7th day after vaccination (Fig. 1, II c). Inhibition of the growth of *M. tuberculosis* was observed in those experiments in which Soton's medium contained serum obtained from guinea pigs 15, 20, and 30 days after immunization (Fig. 1, III c). In cases in which the culture of *M. tuberculosis* was grown in the presence of serum taken on the 40th day after vaccination of the guinea pigs, the degree of depression of multiplication of *M. tuberculosis* was much lower

than when taken earlier. The addition of serum taken from guinea pigs on the 50th and 60th days after vaccination to the Soton's medium had no effect on the proliferation of *M. tuberculosis* (Fig. 1, IV c).

Our results did not conflict with those of Hubacek and Malek [14], who claimed that the bacteriostatic property of the rabbits' serum was not detectable 20 days after immunization. The only difference was that in our experiment the inhibiting action of the guinea pigs' serum disappeared at the end of 40 days after the injection of BCG.

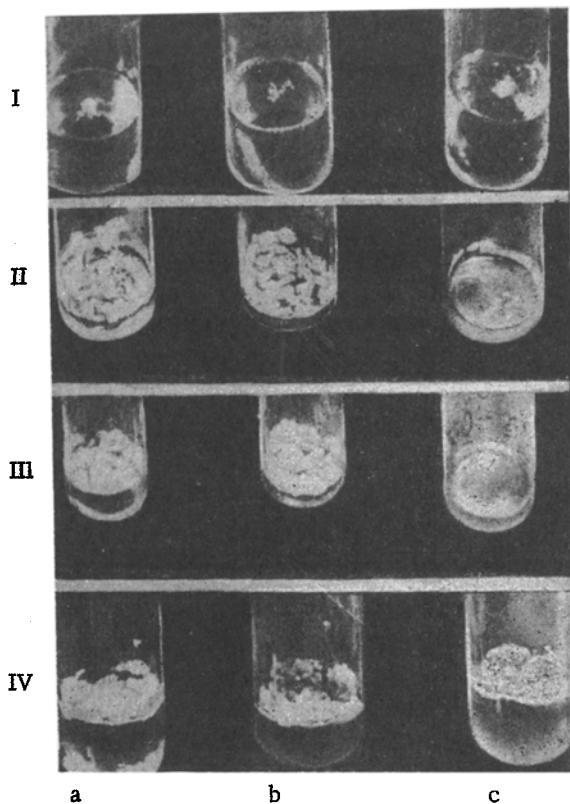


Fig. 1. Multiplication of *M. tuberculosis* in the control series (a) and in the presence of the serum of healthy (b) and immunized (c) guinea pigs. I) At the moment of seeding; II) on the 7th day after immunization; III) on the 30th day; IV) on the 60th day after immunization.

Intact serum from the guinea pigs on the 25th and 40th days after injection of BCG did not alter the bacterial catalase; the same serum, after preliminary heating, lowered the activity of the enzyme. The effect of the untreated serum was evidently the result of the interaction between constituent factors acting in opposite directions.

Our suggestions that two factors are present in the serum of immunized guinea pigs does not conflict with the results of investigations by Vasil'ev [2], who found that the bacteriostatic properties of human serum in relation to various microorganisms can be explained by the presence of two agents: one relatively thermostable (the β -lysin of Peterson), the other thermolabile (identical with complement).

Hence, in the course of the formation of resistance to tuberculosis, the serum of guinea pigs acquires the property of inhibiting the proliferation of *M. tuberculosis* and of modifying the catalase activity of the microorganisms. This property of the serum disappears 40 days after immunization of the animals. The phenomenon we have described may be attributed to the presence of two agents in the serum, one of which is relatively thermostable.

The catalase activity of *M. tuberculosis* was unchanged by the action of the serum of normal guinea pigs. The catalase activity of *M. tuberculosis* cultivated in the presence of the serum of guinea pigs taken on the 40th, 50th, and 60th day after immunization was also indistinguishable from that of the controls. Determination of the catalase activity of *M. tuberculosis* cultivated in medium containing serum from animals in various stages of the development of immunity was not undertaken because of the absence of growth of the microorganisms in these conditions. Experiments were therefore carried out in which *M. tuberculosis* was exposed to the action of the serum for 2 h. These showed that the catalase activity was changed drastically in these circumstances (Fig. 2). The significant variations in the activity of the enzyme demonstrate that the bacterial cell was evidently forced to modify its type of metabolism and to adapt itself to the unfavorable conditions. Our hypothesis is rendered more probable by the fact that Clark [8] observed an increase in the catalase activity in different microorganisms in response to the noxious influences of the external environment.

The factor modifying the catalase activity of *M. tuberculosis* could not be detected in the serum of the guinea pigs on the 40th day after immunization (see Fig. 2), i.e., concurrently with the cessation of the bacteriostatic action.

From the character of the effect of heated serum on the catalase activity of *M. tuberculosis* (Fig. 3), we may conclude that the serum of immunized guinea pigs contains not one, but at least two factors acting on bacterial enzymes. One of these factors is sensitive to heat; its effect is clearly seen on the 20th day after immunization of the animals. The action of the other, relatively thermostable factor was more marked on the subsequent days of the investigation.

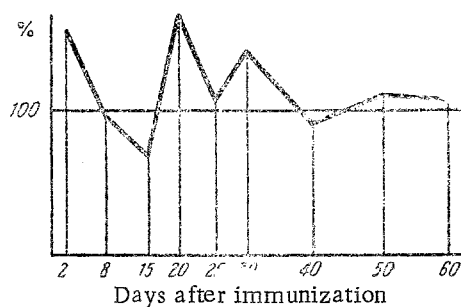


Fig. 2. Changes in the catalase activity of *M. tuberculosis* due to the action of the serum of immunized guinea pigs. The activity of the enzyme in the presence of the serum of intact animals is taken as 100%.

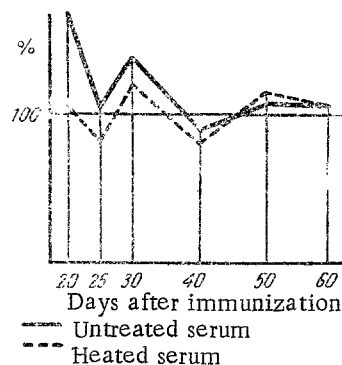


Fig. 3. Action of untreated and heated serum of immunized guinea pigs on the catalase activity of *M. tuberculosis*.

SUMMARY

This work is devoted to the study of the effect produced by the serum of immunized guinea pigs on the multiplication and catalase activity of the tubercle bacillus. As established during the process of immunity formation, the serum of the animals acquires the capacity of inhibiting the growth of the tubercle bacillus and of considerably changing the activity of microbial catalase. Two factors, one of which is relatively thermostable, are evidently the active principles. The described property of the serum disappears 40 days after the immunization.

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