

THE INFLUENCE OF THE PLASMA AND BLOOD SERUM OF ANIMALS OF DIFFERENT SPECIES-RESISTANCE ON THE ASSIMILATION OF OXYGEN AND PHOSPHORUS BY *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis enters the blood vessels very easily, but in the majority (70%) of patients suffering from chronic disseminated tuberculosis they fail to multiply in the blood and are soon eliminated. This is not the case in patients suffering from localized lung lesions [6]. The property of the serum to prevent the multiplication of *M. tuberculosis* is explained [7] by the presence of an inhibitory factor. Some authors [3] believe that the active serum factor in a certain group of patients as well as of animals suffering from tuberculosis or sensitized to tuberculin represents a substance of globulin character; others [5] ascribe this role to the enzyme lysozyme or to a closely related substance. The latter authors emphasize that the sera of immunized rabbits and of a small group of normal persons (10%) also have a bacteriostatic effect upon *M. tuberculosis*.

It seems that not only lysozyme but also many other blood enzymes are capable of affecting *M. tuberculosis*. It is known, for example, that serum lipase affects this organism: in patients in whom the clinical course of the tuberculous process is more favorable the lipase activity is higher than in persons suffering from progressive tuberculosis. Similarly, the aminopeptidase is twice as active in the serum of rabbits infected with tuberculosis as in the serum of non-infected rabbits [2]. Schultz and Weiss [9] found that some sera exerted an increased proteolytic activity, a feature which they regarded as a sign that the person in question was resistant to tuberculosis.

The studies of authors who made attempts to discover antibodies in the serum of patients and animals suffering from tuberculosis are of particular interest. These authors were able to establish the presence of antibodies in the serum of rabbits infected with tuberculosis. These antibodies were frequently present in so small quantities that the serum had to be concentrated before the antibodies could be found. Among 40 patients suffering from tuberculosis investigated, antibodies were found in only five; in a similar group of healthy persons they were found to be absent without exception [1, 8]. Other authors [4] found hemagglutinating antibodies after treating the serum of some patients suffering from tuberculosis with cold ethyl alcohol. Treatment of the serum of healthy persons did not reveal the presence of antibodies.

Notwithstanding the considerable interest of these findings, it can hardly be assumed that antibodies which can be found with such difficulty and in so rare cases can play an appreciable role in the immunity to tuberculosis.

We failed to find reports in the literature directly concerning the role of humoral factors in the natural species-resistance of animals to tuberculosis. In the opinion of Pagel and co-workers [7] the bacteriostatic properties of the serum are not connected with individual or species-specific features of the serum and characterize only certain groups of patients or animals suffering from tuberculosis. At the same time, however, it could be

established in the Laboratory of Microbiology and Morbid Anatomy of our Institute that after intravenous injection of *M. tuberculosis* the organisms rapidly disappear from the blood of white mice, and these findings, as well as the absence of the organisms from the blood of the animals mentioned above after other methods of infection, warrant the assumption that a certain factor exists which prevents the continued survival of the organism in the blood.

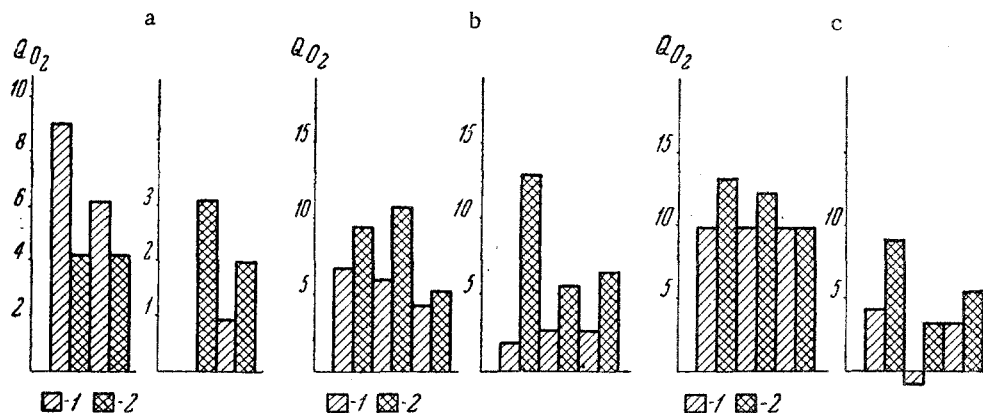


Fig. 1. The influence of mouse plasma upon the absorption of oxygen and phosphorus by *M. tuberculosis* (a); the effect of mouse plasma (1) and rabbit plasma (2) upon the same processes (b); the influence of mouse serum (1) and rabbit serum (2) upon the respiration and phosphorus assimilation by *M. tuberculosis* (c).

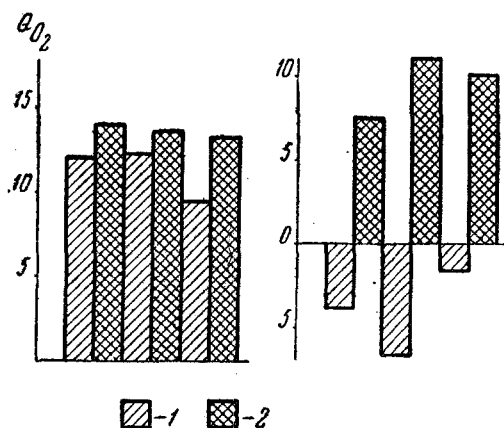


Fig. 2. The effect of serum of immunized (1) and (2) normal guinea pigs upon the respiration and phosphorus assimilation of *M. tuberculosis*.

It was the aim of the present paper to establish some of the biochemical foundations for the influence of the plasma and blood serum of animals possessing a species-resistance of different degree to *M. tuberculosis*. It is well known that all living cells, and in particular bacteria, require for the performance of their vital functions, for the normal course of their plastic metabolism, multiplication, toxin formation, and many other functions, phosphate groups as well as energy; the latter is stored in the high-energy phosphorus compounds which accumulate in aerobic bacteria in the course of oxydative phosphorylation. In view of this fact we thought it worthwhile to establish above all the changes (if any) occurring in the intensity of respiration and assimilation of inorganic phosphate in *M. tuberculosis* in the presence of plasma and serum from different animals.

METHOD AND RESULTS

The plasma and the serum of 542 mice, 23 rabbits and 125 guinea pigs were used for the study. It is well known that mice are relatively resistant to infection with tuberculosis, whereas rabbits, and in particular guinea pigs, are highly sensitive. The experiments were carried out with several strains of *M. tuberculosis* of the bovine type (strain No. 23 of the Leningrad Institute of Tuberculosis, Vallée strain and BCG strain) and one strain of the human type (strain No. 97 of the Leningrad Institute).

The respiration of the bacteria was studied manometrically in a Warburg apparatus. The bacterial suspension in Soton's medium was placed into the containers and the plasma or serum in question was added in volume corresponding to one quarter of the total volume of the mixture. In the control samples a corresponding volume of normal saline was added instead of the serum. The assimilation of phosphorus was judged by the decrease of inorganic phosphate in the medium after incubation in the Warburg apparatus at 37° C for 20 min.

In approximately 73% of cases we were able to observe a marked suppression and sometimes also a complete

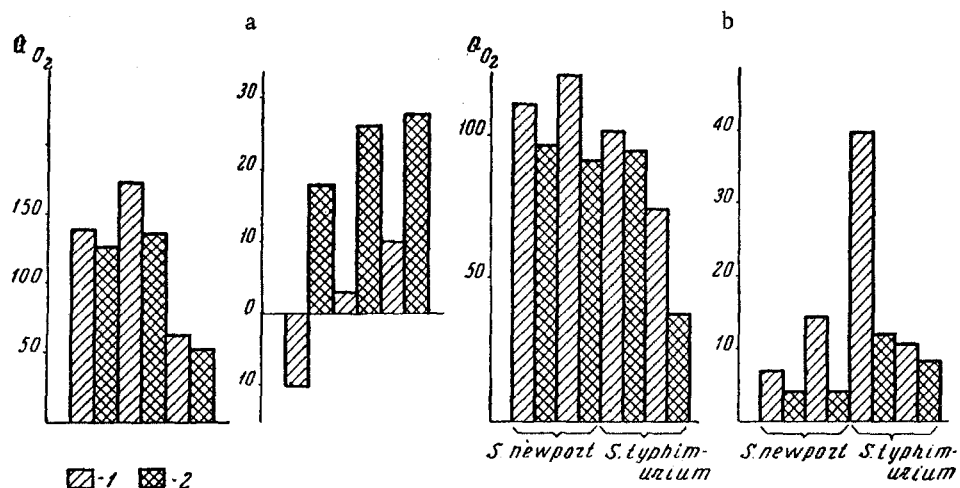


Fig. 3. The influence of mouse serum upon the respiration and assimilation of phosphorus in: a) *E. coli*; b) *S. newport* and *S. typhimurium*; 1) with added serum; 2) without addition of serum.

inhibition of the consumption of inorganic phosphate by *M. tuberculosis* under the influence of mouse plasma, notwithstanding the increased absorption of oxygen. (Fig. 1a). * This could be explained either by disorders of the process of phosphorylation or by an increased breakdown of adenosinetriphosphoric acid. It is obvious that in those cases the energy of respiration cannot be fully exploited by the bacterial cell, a fact which apparently renders their continued existence in the blood of resistant animals more difficult.

In another series of experiments we compared the properties of rabbit and mouse plasma (Fig. 1b). It was found that rabbit plasma exerts a stimulating effect on both the absorption of oxygen and upon the assimilation of phosphorus. In other words the phenomenon observed was exactly the opposite of the phenomenon described above. Similar findings (Fig. 1c) were obtained in experiments in which the plasma was replaced by serum. It seems that in the blood of animals sensitive to tuberculosis, and particularly the blood of rabbits, the bacteria are in more favorable conditions than in the blood of mice. This probably explains the stimulating effect of the addition of blood of some animals to the culture medium upon the multiplication of *M. tuberculosis*.

In our opinion, the results of these two experimental series enable us to assume the existence of a certain factor (or factors) in the blood of animals resistant to tuberculosis which inhibits an extremely important system of biochemical reactions in the microorganisms – a system which represents the basis of their vital activity.

Assuming the capacity of the serum to change the bacterial metabolism in a direction unfavorable from their point of view is connected with phenomena relating to immunity, it could be expected that a similar capacity ought to develop in the serum of sensitive animals as a result of their immunization. To check this assumption we carried out a series of experiments with normal and immunized guinea pigs, respectively. The animals were immunized by intradermal injection of 1 mg dried BCG vaccine in 0.2 ml normal saline. Guinea pigs which gave a well marked Mantoux reaction, read some time after the immunization, were selected for the experiment. It appeared that as a result of the immunization the serum of guinea pigs not only acquires the capacity to suppress completely the fixation of inorganic phosphate, but also the capacity to produce decomposition of the stores of organic phosphorus compounds in the cells, a fact which becomes manifest in the accumulation of mineral phosphorus in the medium (Fig. 2). The character of the action exerted by the blood factor in immunized animals, however, is in some respects different from the character of the factor described above: namely, in the present case no intensification of the oxygen absorption can be observed as it had been observed in the first series; on the contrary: the respiration decreased in all cases to a certain degree. This slightly lower level of the oxidative reactions, however, is nevertheless adequate to secure intensive phosphorylation, a fact which ought to become manifest in the increased consumption of inorganic phosphate. As this does not take place, it must be as-

* Here and in the subsequent Figs., only the results of some of the most typical experiments are given.

sumed that the action of the factor present in the blood of immunized guinea pigs is similar to the factor present in mice, and acts not upon the suppression of the oxygen absorption but directly upon the process of phosphorus esterification.

Our findings are consistent with the facts reported by Myrwik and Weiser [5], which authors showed that the serum of immunized rabbits, unlike the serum of normal animals, has a bacteriostatic effect upon M. tuberculosis. In view of the fact that even a temporary unfavorable influence upon the causative organism may play a part in the host's body's fight against the infection, the findings given above enable us to speak of the presence of a humoral factor in both cases (natural resistance and artificial immunity), a factor which is related to immunity against tuberculosis.

Is the factor present in mouse serum of specific character? To answer this question we investigated the effect of mouse serum upon a number of bacteria in the group of Enterobacteriaceae — Escherichia coli, Salmonella typhimurium (2 strains), Salmonella newport (2 strains) — to some of which the mice are highly sensitive and to others resistant. E. coli is a normal inhabitant of the intestine and does not, under normal conditions, cause any illness in mice, whereas mice are highly susceptible to S. newport, and in particular to S. typhimurium. Using these microorganisms and comparing the effect of mouse serum upon these organisms and upon M. tuberculosis, respectively, we were able to judge the presence or absence of a specific effect of the factor in question.

The results of these experiments, which were carried out in a similar manner as the preceding experiments, revealed that the serum of mice exerts the same effect upon E. coli and upon M. tuberculosis (Fig. 3a), whereas the absorption of oxygen and phosphorus by S. newport and S. typhimurium is stimulated by the same serum (Fig. 3b).

It seems that in these experiments we observed the same phenomenon as in the previous experiment. It is, however, interesting that in the case in question one and the same serum had a different effect upon different microorganisms. In other words the factor present in the serum is neither of universal character nor is it specific: mouse serum exerts the same influence upon E. coli as upon M. tuberculosis.

The results of the investigations carried out by us thus showed that serum of naturally resistant animals is capable of suppressing the oxidative assimilation of phosphorus in M. tuberculosis; this fact suggests the presence of a humoral factor in the animals, which factor is apparently related to phenomena connected with the immunity to tuberculosis. The factor is not specific, as it exerts the same influence upon the phosphorus metabolism of other microorganisms (E. coli).

The serum of immunized guinea pigs acquires the capacity to prevent completely the assimilation of inorganic phosphate by M. tuberculosis.

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SUMMARY

This paper deals with the effect of the blood plasma and serum of animals with different species-resistance to tuberculosis on the respiration and phosphate assimilation in Koch's bacillus. An ability of the serum in the naturally resistant animals to depress the oxidative phosphorus assimilation in M. tuberculosis was detected, which testifies to the presence in these animals of a humoral factor evidently connected with phenomena of antituberculosis immunity. The latter confirms the fact that the serum of immunized guinea pigs acquires an ability to stop the binding of inorganic phosphate by M. tuberculosis. The factor described is not specific, since it exerts the same effect on the phosphorus metabolism of other microorganisms as well (E. coli).

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