USE OF MICROORGANISMS FOR DETERMINATION OF THE PHAGOCYTIC FUNCTION OF THE RETICULOENDOTHELIAL SYSTEM

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The study of the phagocytic function of the reticuloendothelial system (RES) is based on the ability of its cells to take up from the blood microorganisms [12], suspensions of dyes [5-9] or radioactive and other substances [13, 14]. The function is interpreted in terms of the rate of disappearance of the injected substance from the blood.

The phagocytic power of the RES is of special interest in connection with infections and chemotherapy. Evidently recovery from the infectious disease depends not only on the action of the drug on the microorganisms but also on the defensive powers of the organism itself. Here the RES plays an important part.

It is important to know how the drugs used affect the condition of this system. It must be realized that whereas the influence of chemotherapeutic substances and in particular of antibiotics on the activity of the phagocytes of the blood has been quite extensively studied, very little is known about their action on the RES.

There is no doubt that it is not easy to find a method. Also it is very important that the method of determination of the phagocytic power of the RES should measure up to the task in hand, i.e., to a study of the absorption by it of microorganisms.

V. K. Vysokovich has shown that microorganisms injected intravenously soon disappear from the bloodstream on account of their absorption by the RES [1]. Many investigators who have continued this line of research have studied the distribution of bacteria in the body. The work of P. N. Stupnitskii is of special interest; he studied the distribution of plague bacteria in guinea pigs [9, 10], and showed that when the infection is generalized plague bacteria entering the bloodstream collect rapidly and selectively in the spleen and liver, i.e., in organs rich in cells of the RES.

V. K. Vysokovich's discovery has been the starting point from which we have developed our method of studying the power of the RES to phagocytose microorganisms.

Animals were injected intravenously with Staphylococcus aureus (strain 209) in physiological saline. Previously they had been given the substance whose action on the RES was to be investigated. It was found best to inject the preparation at a time such that its concentration was maximal at the moment the staphylococcal injection was given. For rabbits weighing 2-3 kg we recommend the injection of 1 ml of saline containing 2-5 billion bacterial cells per kg weight. For mice weighing 18-20 g it is best to inject 10 million bacterial cells in 0.4-0.5 ml of fluid.

With this number of bacterial cells it is easy to compare the number of colonies in Petri dishes after blood cultures have been made. Portions are explanted 5, 15, and 30 min after the injection of the culture. In rabbits the blood was taken from an ear vein, and in mice it was obtained by decapitation. Portions of 0.02 ml were planted out side by side on two Petri dishes containing a nutritive medium. After 18 h incubation in a thermostat the colonies were counted. As controls we used animals which had not received the preparation but which had been given the bacterial injection. The bacterium we have recommended for test is not the only one which can be used to study the phagocytic power of RES. In principle any other microorganism could be used which gives good colonies when the blood is planted out on a solid nutritive medium.

Influence of Chloramphenicol on Phagocytic Power of the RES of Rabbits, as Determined by the Microorganism Method and Calculation of the Congo-Red Index

Method of determination	Type of investigation	Index
Microorganism method	Experiment	1412 ± 247 (814-2009)
	Control	474 ± 94 (257-691)
Congo-Red index	Experiment	78
	Control ("background")	79

Because of the possibility of a direct action on the microorganisms it is important to find whether the results obtained derive from the influence of the preparation on the RES or from direct action on the bacteria.

To eliminate the antimicrobial action of the preparation bacteria may be used which are resistant to the preparation. However in many cases this is not necessary. We have studied the influence on the RES of chloramphenicol and of compounds of the tetracycline group [2-4] using strains of staphylococcus sensitive to them. The control consisted of the blood of animals treated with the same amount of antibiotic. For this purpose the same culture was added in vitro to the blood of animals which had been treated with the preparations, and samples were explanted into dishes. Blood of untreated animals served as control. No difference was found between the numbers of bacteria in the explants from the two groups.

To compare our method with the usual one in which a Congo-red index is measured we carried out special experiments. We determined the influence of chloramphenicol on the absorptive power of the RES. One dose of 50 mg/kg was given by mouth to seven rabbits; 30 min later an injection was made of Staphylococcus aureus. Seven rabbits of the control group received the staphylococcus only. Five minutes after infection blood was taken from both groups and the phagocytic power of the RES was determined as already described.

The Congo-red index was measured in 20 rabbits. Before the injection of chloramphenicol 2-3 determinations of the "background" were made at intervals of 5-6 days. Then after the last Congo-red determination the animals received 50 mg/kg by mouth, and 30 min later the dye was injected. Samples of blood were taken from the heart before, and 5 and 30 min after, injection of the preparation. The procedure was repeated at least twice at intervals of 5-6 days. The Congo-red determination for this group of animals therefore took about four weeks.

EXPERIMENTAL RESULTS

The results obtained by the microorganism method are shown in the table, and indicate the suppressive influence of chloramphenicol on the RES.

For this purpose the Congo-red index and the control gave the same indices. No statistical method was employed to compare the numbers 78 and 79, because there could be no question of a significant difference. We must note that Chernokhvostova [11] found that injection of as much as 100 mg/kg of Congo-red caused a reduction in the activity of the RES. The use of the microorganism method enabled us to show the corresponding effect more rapidly by the injection of therapeutic amounts of a drug. The results obtained on rabbits have been confirmed in experiments on white mice [2, 3].

We may recommend the microorganism method of determination of the absorptive power of the RES. It is interesting primarily in connection with the influence of drugs on this system.

The advantages are that the results are clear-cut, the experiments can be performed rapidly on small laboratory animals, and this approach is suited to the study of drug action.

SUMMARY

The paper describes a method based on the use of microorganisms for determination of the phagocytic power of the reticuloendothelial system; it is based on recording the number of microorganisms isolated from the blood after intravenous injection. The method is recommended for a study of the effect of drugs on the reticuloendothelial system.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
