

THE CHANGE IN PHAGOCYTIC ACTIVITY AND IN CERTAIN  
CYTOCHEMICAL REACTIONS OF THE LEUKOCYTES  
OF GUINEA-PIG PERITONEAL EXUDATE DURING IMMUNIZATION

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In the reviews by A. D. Ado [1] and Suter [5] which deal with recent problems of phagocytosis, numerous instances are quoted of the close relationship between the phagocytic activity of leukocytes and their metabolism. M. P. Pokrovskaya and her co-workers [2] found that in an immune animal the phagocytic activity of the leukocytes was increased, and that they contained more glycogen. Until now there has been a lack of experimental evidence bearing on this point.

The principal contribution of the research here described has been to determine the effect of immunization on the following properties of the leukocytes: phagocytic activity, stability during reaction with dysenteric bacteria, glycogen content and peroxidase activity.

METHOD

The experiments were performed on 30 guinea-pigs weighing 400-500 g. The leukocytic properties of the peritoneal exudate were examined on six occasions: before vaccination, three days after a second injection of vaccine, and 3, 15, 30, and 60 days after a third vaccine injection. For immunization a tetra-vaccine was used which was obtained from the Gor'kii NIIEG (series 285); three injections of 1.5 ml were given at intervals of 3 days.

To determine the leukocytic reaction, we used peritoneal exudate collected over a period of 18 hours by a method we have described previously [3]. The phagocytic reaction was carried out in a volume of 0.3 ml: to 0.2 ml of exudate were added 0.1 ml of a suspension of a 24-hour culture of e-5033 Flexner dysenteric bacilli containing 0.5 milliard cells per ml. After they had been carefully mixed, the combined suspension was placed in a thermostat at 37°. Three smears were made every ten minutes, for two hours. One smear was fixed in Nikiforov's mixture and stained in Romanowsky-Giemsa and used to determine the phagocytic index (number of bacteria captured per leukocyte); the other two were used for cytochemical analysis.

Experiments were made on 10 guinea-pigs, and the phagocytic reaction was followed during a two-hour incubation period; it was found that the reaction increased during the first 20 minutes and abated in the next 60 min. The maximum reaction occurred after 20 and the minimum after 80 min; therefore, in all subsequent tests observations were made at these two times.

The density of the leukocyte suspension was determined in the usual way by using a hemocytometer. Glycogen was identified by the method of A. L. Shabadash [4], and peroxidase by Graam's method. After 100 leukocytes had been counted they were divided into three groups according to the number of granules they contained (+, ++, +++). In compiling the results, only leukocytes marked ++, or +++ were counted as containing active peroxidase and glycogen.

All the results were treated statistically, and the mean value  $M$ , the standard deviation  $\sigma$ , the square of the standard error of the mean  $m$ , and the standard error  $t$  were calculated.

## RESULTS

Through having studied the phagocytic reaction we were able to make the counts after 20 minutes incubation, at the time when the changes were best shown (Table 1).

During the vaccination period there was a gradual increase in the number of phagocytes. At the end of the course, on the third day, it was maximal and equal to twice the original value.

Less clearly marked changes were observed when the number of phagocytes was counted after 80 minutes. At all times during the vaccination course the number obtained in this count was less than after 20 minutes incubation, and the difference between the 20-minute and 80-minute counts increased; three days after the course it was 11 times greater than before immunization had begun. The increase in the difference gives some indication of the increase in the rate of destruction of bacteria within the leukocytes.

TABLE 1. Change in the Number of Phagocytes during Guinea-pig Immunization

No. of phagocytes	Before immunization	3 days after a second injection of vaccine	At the end of immunization			
			after 3 days	after 15 days	after 1 month	after 2 months
After 20 min incubation	0.82	1.68	1.89	1.65	1.23	1.04
After 80 min incubation	0.74	0.99	1.01	1.04	1.01	0.92

TABLE 2. Change in the Number of Leukocytes containing Active Peroxidase and Glycogen during Guinea-pig Immunization

No. of leukocytes %	Before immunization	3 days after a second injection of vaccine	At the end of immunization			
			after 3 days	after 15 days	after 1 month	after 2 months
Containing active peroxidase	63	75	83	77	65	64
Containing glycogen	77	84	87	85	83	78

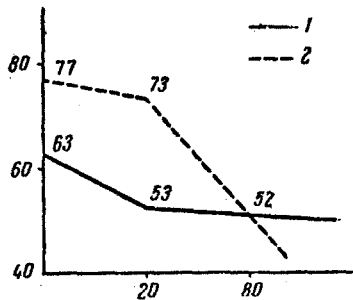
The resistance of the leukocytes to the Flexner bacilli was assessed from the number destroyed, i.e. from the reduction in the number present in a given volume. The rate of destruction of the leukocytes mixed with the bacilli was compared with the rate in a control tube containing no bacilli. The leukocyte concentrations were measured before and after an 80-minute period of phagocytosis. It was found that in the nonimmune animals contact with the bacilli caused a four-fold increase in white cell destruction, and in the immune group the increase was only two-fold.

Resistance to the dysenteric bacilli was therefore doubled by immunization.

The results for peroxidase activity and glycogen content are shown in Table 2.

It can be seen from Table 2 that the number of leukocytes containing active peroxidase and glycogen gradually increased during immunization. Three days after the treatment had begun the number containing peroxidase had increased by 20%, and there was a 10% increase in those which contained glycogen.

The figure shows the histochemical changes in the nonimmune group.



Change in the number of leukocytes containing active peroxidase and glycogen during phagocytosis. 1) Number of leukocytes containing peroxidase; 2) number of leukocytes containing glycogen.

In the first 20 minutes the number of leukocytes containing glycogen and active peroxidase was reduced, and the reduction in the latter was the more marked. After 80 minutes there was no further reduction in the number of cells containing active peroxidase, but those containing glycogen suffered an even greater reduction. It would appear therefore that intracellular peroxidase plays a part in the initial stage of phagocytosis, and glycogen is involved in the final phase.

#### SUMMARY

The phagocytic activity of the peritoneal exudate leukocytes increased during the process of guinea pig immunization with dysentery vaccines. There was a simultaneous rise of the leukocyte resistance to the injurious effect of dysentery bacilli. A rise of leukocyte phagocytic activity was associated with some changes in their metabolism. The phagocytic number is seen to rise to the maximal level on the third post-immunization day; the number of leukocytes containing active peroxidase and glycogen also showed a maximal increase. During phagocytosis, the number of leukocytes containing glycogen and active peroxidase becomes reduced. Cytochemical methods used in studying

phagocytosis permit a closer approach to the physiological characteristics of the process.

#### LITERATURE CITED

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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.