

STIMULATION OF PHAGOCYTOSIS IN VITRO BY THE ACTION OF A PHYTOAGGLUTININ OBTAINED FROM THE SEEDS OF *Phaseolus vulgaris*

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UDC 612.112.3-014.46:
615.32 :582.736

Several authors have demonstrated the effect of phytoagglutinin, as also of other antigens, on the division of the leukocytes in human peripheral blood [7-11]. For this reason, it may be postulated that phytoagglutinin may stimulate mitosis not only in the leukocytes of the peripheral blood, but also in the cells of certain other tissues retaining the ability to divide and to react immunologically. The authors' hypothesis has been confirmed in experiments on guinea pigs [4], and also in clinical conditions [6]. In these investigations stimulation of tissue immunity was observed under the influence of phytoagglutinin.

In the present investigation the action of a phytoagglutinin from the seeds of *Phaseolus vulgaris* was studied on phagocytosis in vitro.

EXPERIMENTAL METHOD

The phytoagglutinin (the preparation Phaseolosaxin) obtained by one of the authors [3] was used in the experiments.

The investigations were carried out in vitro on leukocytes of human blood taken from 10 healthy persons. The method of Wright and Douglas, modified by Velchanov [1], was used in the investigation. To study the phagocytic activity of the leukocytes, a living bacterial culture of *Staphylococcus aureus* (strain No. 209), grown for 24 h in test tubes on solid agar, was used. The density of the bacterial suspension varied from 1-2 billion cells/ml. Phagocytosis was stimulated by using various concentrations of phytoagglutinin.

The phagocytic index (M) — mean number of bacteria ingested by one leukocyte — was determined after counting 100 uninjured leukocytes among which some cells had ingested bacteria while others had not. To express the phagocytic activity as a percentage, the results of the control experiments were taken as 100.

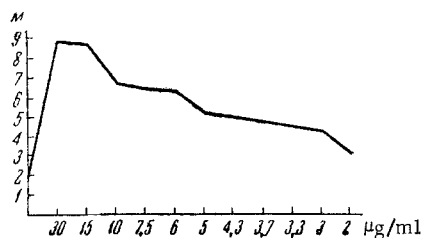


Fig. 1. Phagocytic index (M) of leukocytes treated with phytoagglutinin in vitro.

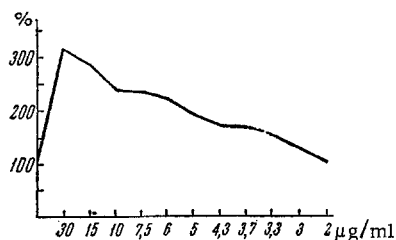


Fig. 2. Phagocytic activity of leukocytes treated with phytoagglutinin in vitro (aggregated curve from results of ten experiments).

EXPERIMENTAL RESULTS

The experimental results are illustrated in Figs. 1 and 2.

It is clear from Figs. 1 and 2 that in all the investigated blood samples from the 10 persons, a considerable increase in the phagocytic activity of the leukocytes from 233 to 474% was observed (mean 332%) during the action of phytoagglutinin.

The curve of the phagocytic activity of the leukocytes shown in Fig. 1 is based on the absolute values of the phagocytic index in one of the experiments. This curve is very similar to the curves of the remaining experiments. The aggregated curves of stimulation of phagocytosis based on the results of all 10 experiments is shown in Fig. 2. It is clear from Figs. 1 and 2 that the curve of phagocytic activity had a maximum in the initial dilution of phytoagglutinin ($30 \mu\text{g/ml}$), after which it fell. This fall in some cases reached the control level at a phytoagglutinin concentration of $4.3 \mu\text{g/ml}$, and in other cases at higher dilutions of the preparation.

The polymorphonuclear leukocytes in some cases were injured, and in films made from them they were fewer than usual. Polymorphonuclear leukocytes in cultures of peripheral blood die early. When the experiments were carried out in vivo, and the phytoagglutinin injected parenterally into rabbits, a decrease in number and degeneration of the polymorphonuclear leukocytes were also observed [5].

An increase in the phagocytic activity of the mononuclear cells was also observed under the influence of phytoagglutinins. Besides monocytes, a certain proportion of the lymphocytes takes part in phagocytosis (Fig. 3a). In control experiments, no lymphocytes were found to be performing phagocytosis. The phenomenon described above shows that phytoagglutinin can stimulate

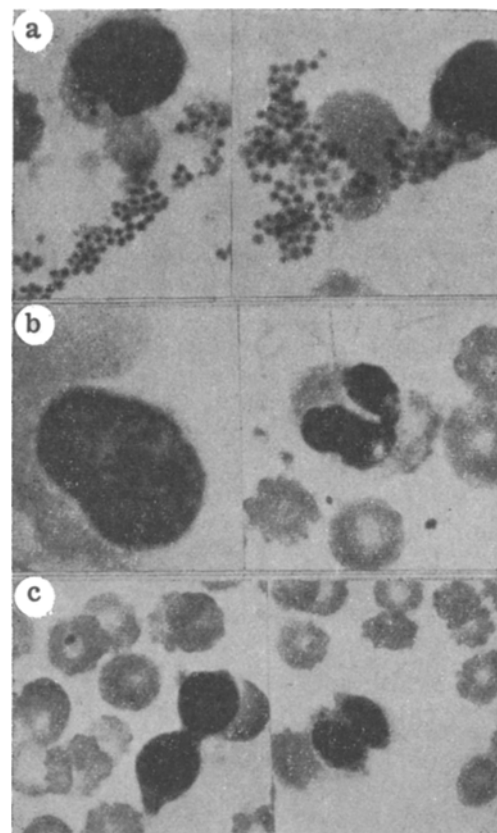


Fig. 3. Leukocytes of human peripheral blood treated with phytoagglutinin in vitro. a) Lymphocytes (vacuoles with bacteria contained in them are seen in the cytoplasm); b) a monocyte and a polymorphonuclear cell (vacuoles, containing bacteria can be seen in the nuclei of the cells); c) amitosis in lymphocytes. Romanovsky-Giemsa. Objective 100, ocular 10.

the phagocytic activity of lymphocytes which, in ordinary conditions, do not possess this activity. Phagocytosis was observed in the nuclei of the monocytes and polymorphonuclear cells (Fig. 3b) – a phenomenon described also by other authors [2].

In the present experiment the following fact was interesting. In blood films from some persons, with dilutions of phytoagglutinin of 7.5 and $6 \mu\text{g/ml}$, mononuclear leukocytes were observed in a state of division (Fig. 3c).

Hence phytoagglutinin, in certain concentrations, is a powerful stimulator of phagocytosis in all leukocytes. This property of phytoagglutinin may be explained by its marked antigenic activity. As a result of investigations of the antigenic structure of phytoagglutinin by the method of double diffusion in agar it has been found that this substance contains 12 antigens [12].

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