

## INHIBITION OF MIGRATION OF THE BLOOD LEUKOCYTES IN GUINEA PIGS WITH HYPERSENSITIVITY OF DELAYED TYPE TO FOREIGN TISSUE ANTIGEN

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Inhibition of migration of the blood leukocytes was studied in guinea pigs sensitized with rabbit kidney tissue extract (dose 10  $\mu$ g and 6.5 mg). Observations on leukocyte migration were made in Perfil'ev-Gabe rectangular capillaries. Immunologically, the phenomenon was distinctly specific in character. In the experimental model used the phenomenon of inhibition of leukocyte migration developed later than the positive allergic skin tests and was most marked 4-8 weeks after the beginning of sensitization. The use of heparinized blood as substrate for the investigation by the method used has many advantages over the original method and its modifications.

Investigations have shown [5-7] that the activity of leukocytes obtained from peritoneal exudate or lymph glands of animals with hypersensitivity of delayed type to migrate toward an antigen is inhibited by the addition of this antigen to the surrounding medium, which is not observed if any other antigens are added instead or in experiments with cells from unsensitized animals. This phenomenon is regarded as a reaction of hypersensitivity of delayed type *in vitro*. However, considerable difficulties arise during its study and use, even in experimental conditions, not to mention in clinical practice.

This paper describes the results of investigations in which, by the use of a new and comparatively simple method, definite and reproducible results can be obtained during repeated tests in experiments of various kinds on large numbers of animals.

### EXPERIMENTAL METHOD

Intact guinea pigs and guinea pigs sensitized with tissue antigen, in the form of an extract of rabbit kidney prepared by Witebsky's method, with a protein content of 30 mg/ml were used. The antigen was diluted in physiological saline; the appropriate dilution was mixed in equal volumes with Freund's complete adjuvant and injected into guinea pigs in a dose of 0.1 ml of the mixture into the plantar pad. The guinea pigs of one group were sensitized with a dose of 10  $\mu$ g protein, those of the other group with a dose of 6.5 mg. To investigate migration of the leukocytes small samples of blood were taken from the heart, mixed with heparin (15 units to 1 ml blood), placed on silicone-treated watch glasses in a volume of 0.2 ml, and treated by the addition of 0.05 ml of various antigens (experimental, i.e., rabbit kidney extract or control, consisting of normal rabbit serum) or Eagle's medium (control), which was also used to prepare the dilutions of antigen for addition to the blood. One end of a plate containing five parallel rectangular channels with a cross section of 0.4  $\times$  0.15 mm (Perfil'ev-Gabe capillary tubes [3, 4]) was immersed in each mixture, so that the tubes filled quickly and uniformly, after which they were sealed at both ends with molten sealing wax and centrifuged for 5 min at 500 rpm. As a result, the leukocytes separated as a layer covering 2-3 micrometer scale divisions (examined with the MBS-2 microscope; ocular 8, objective 0.6) above the larger layer of erythrocytes. The capillary plates were incubated at 37°C for 18-24 h, after which the dimensions of the

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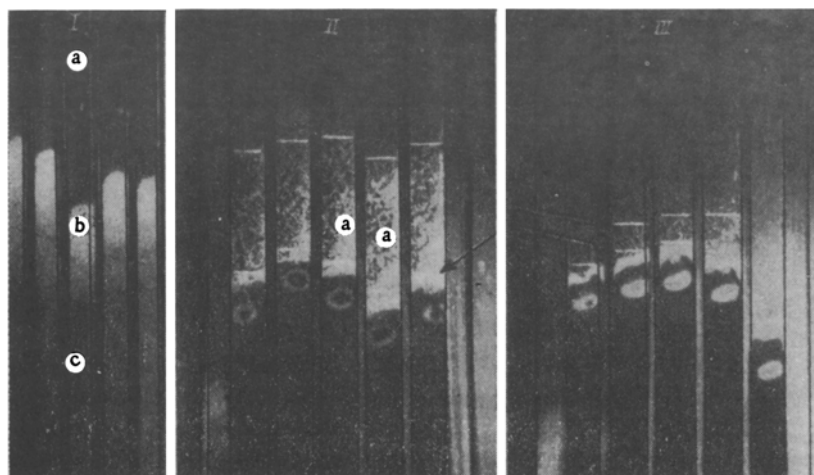


Fig. 1. Inhibition of migration of blood leukocytes in Perfil'ev-Gabe rectangular capillary tubes: I) arrangement of cells in capillary tubes after centrifugation and before incubation: a) lumen of capillary tube; b) layer of leukocytes; c) layer of erythrocytes; II) arrangement of cells after incubation: a) concentrations of migrating leukocytes (pale areas); III) marked inhibition of migration of cells by specific antigen: d) arrows indicate limits of migration of leukocytes.

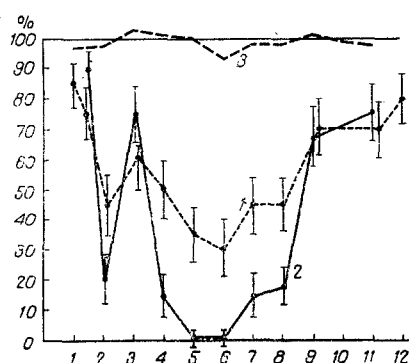


Fig. 2. Change in migration activity of blood leukocytes of sensitized animals under the influence of specific and nonspecific antigens: 1) concentration of specific antigen 1 mg/ml blood; 2) concentration of specific antigen 4 mg/ml blood; 3) concentration of control antigen 4 mg/ml blood. Abscissa, time after sensitization of animals (in weeks); ordinate, degree of migration of leukocytes (in % of control — migration of leukocytes without addition of antigen).

in the mean index of migration (especially if a large dose of antigen was used). Since it was intended to carry out dynamic investigations, the effect of repeated blood sampling on the animals on leukocyte migration was studied. Experiments showed that the mean index of migration for 49 samples taken once a week from seven guinea pigs reach  $7.8 \pm 0.72$ ; i.e., it was greater than the index for single samples. Repeated blood sampling by cardiac puncture thus stimulates migration of the leukocytes.

migration zone of the leukocytes were noted (in micrometer scale divisions), counting from their original position to the limit of migration of the main body of the cells. For each sample studied the mean value of the migration in each of the five capillary tubes was determined. To compare the experimental and control data the following conventional scheme was adopted: differences of less than 30% in the size of the migration zones were disregarded; differences of 31-49% were assessed as weak inhibition (or weak stimulation, if the migration zone in the experimental series was greater than in the control); differences of 50% or more were regarded as showing a marked effect (Fig. 1).

## EXPERIMENTAL RESULTS

1. Indices of Migration of Blood Leukocytes of Intact Guinea Pigs, Effect of Certain Antigens and of Repeated Blood Sampling on It. Blood samples from 34 guinea pigs were studied. The mean index of migration of the leukocytes for the whole group of animals was  $5.8 \pm 0.72$  divisions. Considerable deviation of the individual migration from the mean index occurred in four cases (2.2, 2.5, 11.5, and 13.2 divisions). In all other cases (88%) the individual indices of migration were close to the mean index of migration of the leukocytes. The addition of protein antigens (normal rabbit serum or kidney extract) to the blood samples for testing, in a dose of 1-4 mg/ml, led to weak inhibition of migration of the leukocytes in only one case. As a rule, stimulation of migration was observed and was more marked in the experiments with kidney antigen. In that case there was actually an increase

2. Experiments with Blood Leukocytes of Sensitized Guinea Pigs. A. Effect of Repeated Blood Sampling on Migration of Leukocytes of Sensitized Guinea Pigs. Altogether 100 samples obtained by repeated puncture at intervals of 1 or 2 weeks from 20 guinea pigs sensitized with 10  $\mu$ g antigen and 78 samples from 11 animals sensitized with 6.5 mg antigen were tested. The mean indices of migration were  $8.1 \pm 0.28$  in both cases, i.e., much higher than the mean index for unsensitized guinea pigs after a single sample. Stimulation of leukocyte migration was observed in sensitized guinea pigs throughout the period of observation (17 weeks), starting with the first blood sample (1 week after sensitization), whereas in analogous experiments with intact animals it appeared only after the third or fourth blood sampling and continued for a shorter time (9 weeks).

B. Specific Inhibition of Migration of Blood Leukocytes of Sensitized Animals by Addition of the Corresponding Antigen. Altogether 86 blood samples were obtained in 17 weeks from 13 guinea pigs sensitized with 10  $\mu$ g kidney antigen. Addition of kidney antigen in a dose of 4 mg/ml inhibited migration in 39 samples (43%), and in 22 (24.4%) of them the inhibition of migration was marked; i.e., migration of the cells on the addition of specific antigen to them was inhibited by 50% or more compared with migration of cells of the same blood sample in the control capillary tubes without addition of the antigen. Addition of the control antigen in the same dose inhibited migration of the leukocytes in only nine samples, and marked inhibition was obtained in only two samples. Investigation of 67 blood samples obtained at various times from 11 guinea pigs sensitized with 6.5 mg antigen revealed inhibition of leukocyte migration after addition of kidney antigen in 43 samples (64%), in 30 of which (44.8%) the degree of inhibition was marked. In capillary tubes with the control antigen only weak inhibition was observed in four samples. On the whole, during the investigation of 153 blood samples inhibition of migration of leukocytes was found on the addition of the corresponding antigen in 82 samples (54%), in 52 of which (34%) the degree of inhibition was marked. Addition of the control antigen led to inhibition in only 13 samples (8.5%), to a marked degree in only two (1.3%). The dynamics of the changes in the mean index of migration of the blood leukocytes of the experimental animals after contact with kidney antigen compared with the control value, taken as 100% for each time, is illustrated in Fig. 2. The most marked inhibition of leukocyte migration was found in samples taken 4-8 weeks after sensitization, and it was particularly considerable after sensitization with a large dose of tissue antigen: in that case migration of the cells was completely absent in samples taken on the 35th-42nd day after sensitization.

C. Relations between Specific Inhibition of Migration of Leukocytes and Allergic Skin Tests. It followed from previous investigations [1, 2] that allergic skin tests were observed in dogs sensitized with tissue antigen in doses of between 20  $\mu$ g and 20 mg, with effect from the 5th-6th day after sensitization and they could be reproduced for 2.5-3 months. An increase in the sensitizing dose did not lengthen the period of reproduction of the skin reactions, but it modified their character: if reproduced 8-10 weeks after sensitization they were mixed in character; i.e., besides features of reactions of delayed type they also included features of reactions of immediate type. This conclusion was confirmed by experiments to study the passive transfer of hypersensitivity of the different types, which at this time could be done separately by the cells or serum from the corresponding donors: the cells transmitted hypersensitivity of delayed type and the serum hypersensitivity of immediate type. Comparison of these results with the dynamics of inhibition of leukocyte migration (Fig. 2) showed, first, that this inhibition is delayed by comparison with the allergic skin reaction. Further, maximal inhibition of leukocyte migration on sensitization with a large dose was observed before the times of appearance of allergic reactions of mixed type. Finally, maximal inhibition was obtained after sensitization with a small dose at the same times as after sensitization with a large dose, while the animals preserved hypersensitivity of delayed type in the pure form.

In the analysis of the experimental results it will first be noted that the migrating cells themselves (as has been shown for macrophages of the peritoneal exudate) are only indicator cells in this phenomenon, with no immunological specificity. The inhibition of migration was due to the action of a substance (the migration inhibiting factor) secreted by sensitized lymphocytes on contact with the specific antigen. The indicator cells (in experiments with peritoneal exudate cells) are considered to be macrophages. The experiments described above confirm data in the literature [8-10] that blood leukocytes can perform the same role. A closer comparative study of this problem is required. The next task is to quantify the phenomenon of inhibition of leukocyte migration and, in particular, to determine whether the inhibition factor, in doses smaller than inhibitory, can instead stimulate migration of the leukocytes (as was observed in the controls) and whether the gap between the two types of doses is large enough to warrant description as a "qualitative jump." In experiments with blood cells it is important to exclude any possible effect of interaction between specific

antibodies and the added antigen on the result. In this respect it must be emphasized that in the present experiments sensitization of guinea pigs with a dose of  $10\text{ }\mu\text{g}$  antigen led to a pure form of hypersensitivity of delayed type, and that no circulating antibodies were found throughout the period of observation. So far as animals sensitized in a dose of 6.5 mg are concerned, as was stated above, maximal inhibition of migration was observed in them on the 35th-42nd day, i.e., long before the appearance of allergic skin reactions of mixed type. Confirmatory experiments also were carried out. They showed that the addition of one of the immune sera and the corresponding antigen to normal blood separately or together did not lead to inhibition of leukocyte migration. All these observations indicate that the phenomenon observed in fact reflects hypersensitivity of delayed type, although this fact was not established unequivocally in this investigation. From the technical point of view the advantage of the method used over that described in the literature must be stressed: its simplicity, the small quantity of test material required, the ability to make observations on several samples simultaneously, convenience of quantitative assessment of the results and, finally, the prospects for its use under clinical conditions.

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