AUTOMATIC RECORDING AND ANALYSIS OF LEUKOCYTE MIGRATION BASED ON AN IMAGE ANALYSIS SYSTEM

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In order to assess the state of cellular immunity in clinical practice integral characteristics of mobility and chemotaxis of the leukocytes are frequently recorded. The methods of movement beneath agarose [1], dispersal from a capillary tube [2], and migration through millipore filters [3] are the methods most widely used. The essence of these methods is that mobility of the leukocytes is estimated by migration of their leading edge, i.e., relative to the most mobile cells of the population. With this approach it is impossible to judge the behavior of the more immobile part of the cells. Integral methods give no idea of the scatter of mobilities within the population, or on the character of individual behavior of the cells. Known methods of studying individual migration of cells [4, 5] are very laborious and do not give a complete idea about the whole population. The method we devised combines the advantages of methods of studying both integral and individual mobilities. The method is based on investigation of individual mobility of each cell, followed by statistical analysis of the behavior of a large number of cells, and it gives a complete picture of behavior of the population as a whole.

A problem as difficult as the identification of migrating cells can be solved by the use of specialized computer systems for image analysis. The "Magiscan 2A" image analysis system (England) is suitable for this purpose [6]. Hitherto not only were there no programs capable of solving this problem, but neither were there algorithms on the basis of which suitable programs could be realized. We have developed an algorithm for identifying cells in the course of their migration, and providing different kinds of information about movement of the cells with temporal resolution of not less than 15 sec. The program thus developed remembers the position of the centers of mass of the cells and was able to track displacement of the center of mass of each cell in the field of vision frame by frame. The program can be conventionally divided into two parts: 1) isolation of the cells in the field of vision with the aid of the standard mathematical software of the Magiscan 2A [6], 2) identification of the cells between frames (determination of agreement between the numbers of the cells in the current and preceding frames). Isolation of the cells includes memorizing the image within an assigned time interval, elimination of irrelevant objects (erythrocytes, scratches on the preparation), and memorizing the positions of the centers of mass. Identification between two frames enables shifts of the centers of mass to be calculated, whereas identification during three frames enables a change in the direction of movement to be determined.

The method has been tested during the study of migration of human neutrophils. We used the known method of isolation leukocytes from a drop of blood taken from the finger of a healthy blood donor [4, 5]. Migration of leukocytes on the slide was observed in Hanks' solution with 0.5% human serum albumin ("Serva," West Germany), at a constant temperature of 37°C. The dark-field image obtained on a microscope ("Jena-Mikroskop 250-CF," East Germany), using a 6.3 times objective (frame size 512 × 512 μm), was led to the television camera of the Magiscan, and stored in the Magiscan 2A image analysis system, with intervals of 1 min between frames in this case. By moving the preparation an area with uniform distribution of the cells could be chosen, in which the number of neutrophils in the field of vision did not exceed 70.

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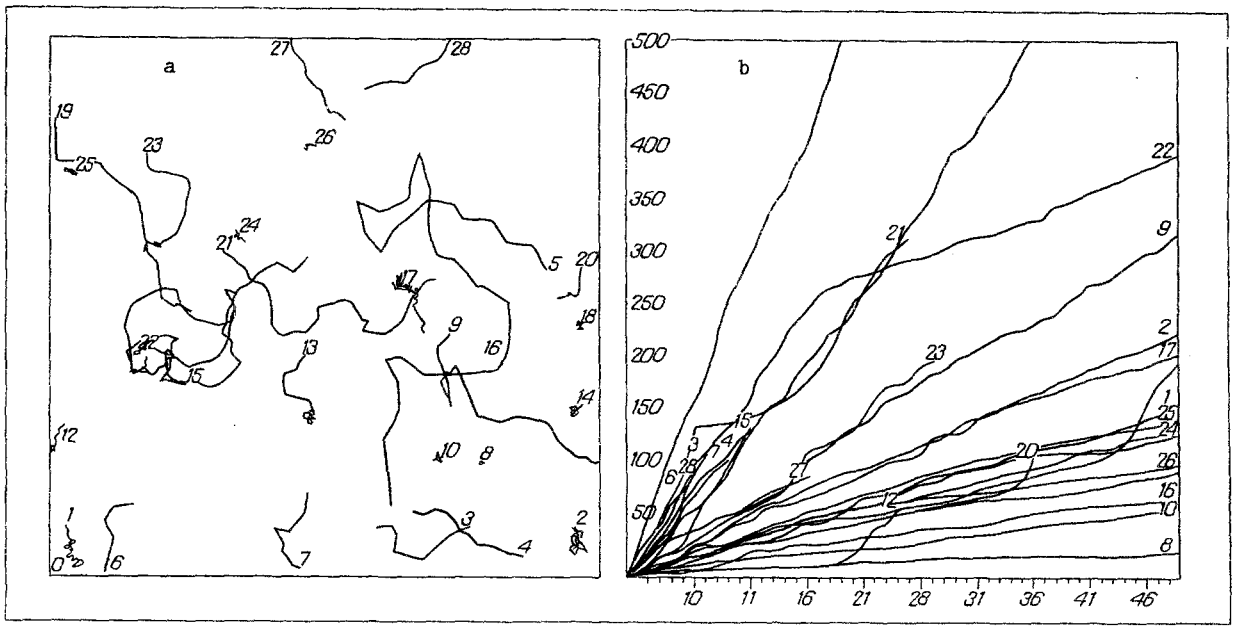

Fig. 1. Trajectories of movement of neutrophils (a) and dependence of path of each cell on time (b). a) Trajectories of movement of neutrophils over 50 frames with interval of 1 min between frames. Cells appearing in field of vision from 2nd to 50th pairs are not shown; size of frame (field of vision) 512 × 512 μm; b) graph showing dependence of path traveled by each cell on time. Abscissa, time (in min, frames); ordinate, path traveled (in μm).

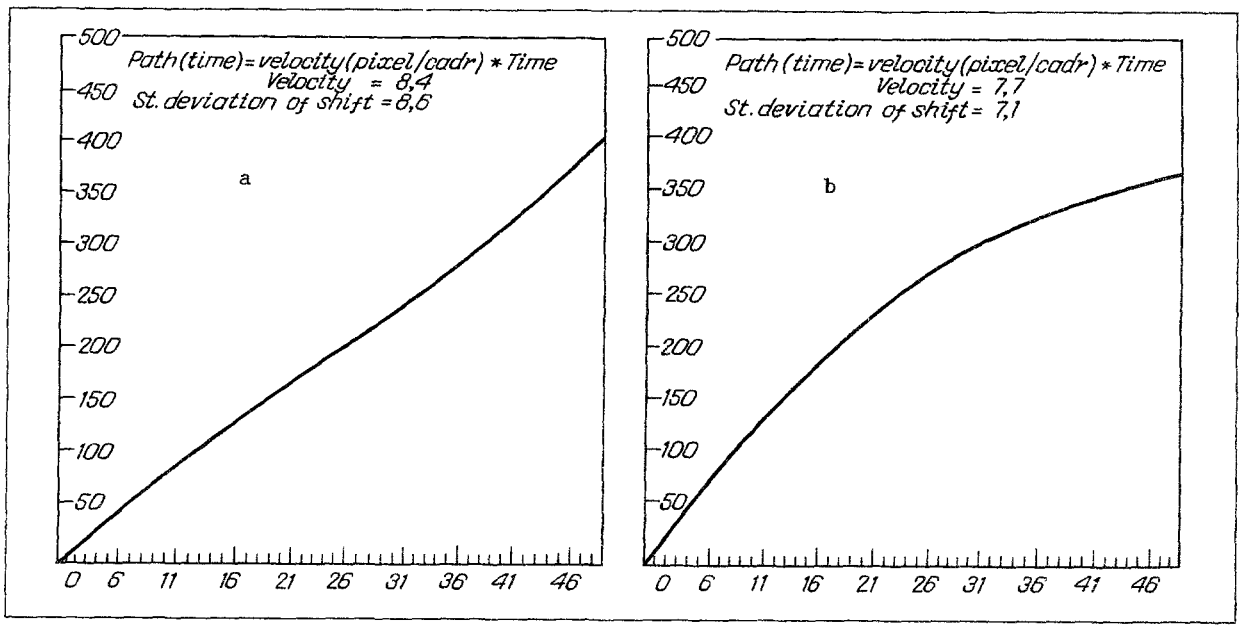

Fig. 2. Dependence of mean path traveled by neutrophils during 50 min on time. a) Normal. Mean velocity (in μm/min) and standard deviation of velocity shown in top part of figure; b) action of UV irradiation. Slow decrease in velocity is evidence of injury to cells.

A histogram of distribution of shifts of the centers of mass in the course 1 min is illustrated in Fig. 3a. The curve resembles the distribution of velocities of brownian particles (the Rayleigh distribution). The mean shift of brownian particles is known to be proportional to the square root of time. Spreading of the leading edge of migration of the leukocytes population, determined by integral methods, in which a long time is required in order to obtain a reliable macroscopic picture, obeys this

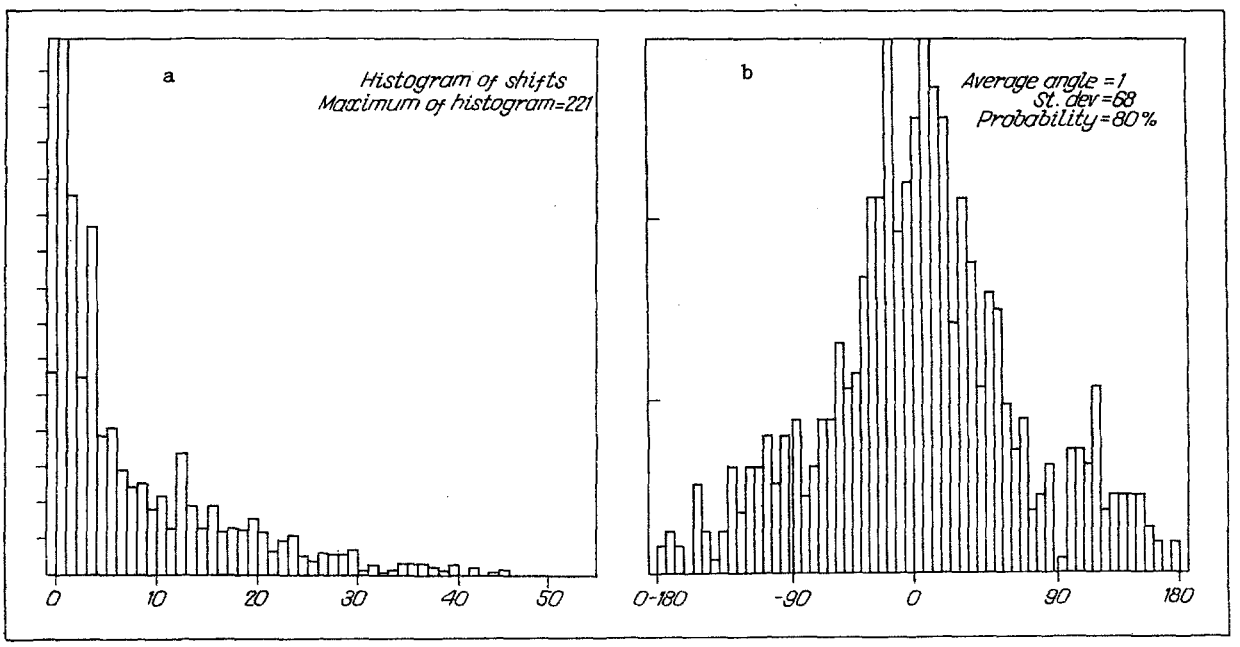

Fig. 3. Histogram of distribution of 1-min shifts (a) and changes in angles of displacement (b). a) Histogram plotted for 50 frames: abscissa, value of shift (in μm); ordinate, number of shifts; b) abscissa, change in angle (in deg); ordinate, number of angles. Probability of angles in region of $\pm 90°$ is 80%.

same rule. With the suggested method statistical significance can be reached within 2-3 min of recording, a histogram of distribution of shifts can be constructed, and the mean shift and standard deviation calculated. The standard deviation shows the true dispersion of activity of neutrophils within the population, for the velocity of movement of each cell is a more or less constant value. The relative numbers of active and passive cells can be determined directly from the appearance of the histogram of their shifts (provided that the velocities of the cells remain constant).

To study intracellular anisotropy connected with the formation of pseudopodia during wandering movement, the angle between two consecutive vectors of shifts (every 3 frames) was calculated and the accumulated data represented by histograms of distribution of angles (Fig. 3b). It will be clear from Fig. 3b that normally, during wandering movement, the dominant angles are $\pm 45°$. Summation of angles within the $\pm 90°$ range, i.e., distinguishing those cells which move forward relative to the previous shift, shows that they account for 80% of the total number. This fact suggests that during an interval of 1 min a pseudopodium is formed mainly at the front end of the cell, or that the time of reorientation of the cell exceeds 1 min. Under conditions of chemotaxis, an oriented movement takes place against a background of chaotic wanderings. This spatial anisotropy of motion can be distinguished by constructing a histogram of the distribution of angles formed by the vector of shift of a neutrophil and chosen axis of coordinates. The suggested method can thus be used to investigate chemotaxis.

By means of this method observations can be made simultaneously on 70 cells, and this guarantees a sufficiently complete statistical picture of their behavior.

Automatic analysis of the results reduces the time of the investigation from preparation of the specimen to printing out of the data to 1-1.5 h.

With this method not only can activity of leukocytes be tracked for a long time under the influence of various factors, but a statistically significant express diagnosis of activity can be obtained within 2-3 min. The time required for express diagnosis is determined by the time taken to prepare the specimen (15-20 min).

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BINDING OF ^{3}H-DOPAMINE AND ^{3}H-QUINUCLIDINYL BENZYLATE BY TISSUE OF AUTONOMIC GANGLIA OF RATS OF DIFFERENT AGES

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Synaptic transmission in the autonomic ganglion, effected by acetylcholine, has several control systems. Some of the slow postsynaptic effects are mediated by muscarinic (M) acetylcholine receptors, some by receptors of transmission modulators. Of these, particular attention is being paid to the role of dopamine receptors as the most likely mediator secreted by the small intensively fluorescent cells of ganelia [1]. The system of M cholinergic reception in autonomic ganglia of adult animals and man have been characterized in detail with respect to parameters of binding of various ligands [6, 7]. Age changes in M cholinergic reception and dopamine reception for ganglion tissues have virtually not been studied.

The aim of the investigation was to study dependence of binding of labeled ligands with M acetcholine and dopamine receptors of ganglion cells on the period of postnatal development of the rat, chosen in accordance with the periods of formation, stable functioning, and age changes in the functional properties of nerve ganglia.

EXPERIMENTAL METHOD

Tissue of the inferior ganglion of the vagus nerve, the lumbar sympathetic ganglia, the great pelvic ganglion (in males), and the paracervical ganglion (in females) of rats aged 1, 7, 14, 28, and 60 days and 24-30 months, with 6 to 25 animals in the group, was used as the test object. Altogether 106 rats were used. Under pentobarbital anesthesia (40 mg/kg) the nerve ganglia of the animals were excised, weighed, and frozen. Serial frozen sections through the ganglia about 15 μm thick were mounted on slides and covered with gelatin. The mounted sections were kept for 24 h at −4°C and then preincubated in buffer solution for 15 min at room temperature. The incubation medium for binding of ^{3}H-quinuclidinyl benzylate (^{3}H-QNB, specific radioactivity 30 Ci/mmole) contained 0.9% NaCl in 15 mM phosphate buffer, pH 7.0. The duration of incubation was 2 h in darkness at 20°C and the concentration of the ligand was 1 nM. The medium for dopamine binding (^{3}H-DA, specific radioactivity 49.7 Ci/mmole) included 0.1% ascorbic acid, 0.1 pM pyrocatecol, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ in 50 mM phosphate buffer, pH 7.7. The incubation time was 1 h at 20°C and the concentration of the ligand 1 nM. In preliminary experiments on membrane preprations of tissue from lumbar ganglia of the sympathetic trunk, the basic parameters of binding were established with the use of atropine (1 μM) and dopamine (1 μM) as displacing agents. The slides with the sections, after the binding procedure, were washed twice in cold buffer for 5 min each time, after which they were placed for a few seconds in distilled water, dried, and put into flasks with toluene scintillator. After 24 h the intensity of counting was recorded on an SL-30 scintillation counter. The results were expressed relative to wet weight of ganglion tissue. The results were subjected to statistical analysis by a standard program package.

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