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UNIFORM LATERAL ORIENTATION, CAUSED BY FLOW FORCES, OF FLAT PARTICLES IN FLOW-THROUGH SYSTEMS

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Recently, it was shown that the lateral orientation of sperm cells disturbs the deoxyribonucleic acid distribution measured by fluorescence in a laterally laser-illuminated flow system. The present results show how flat particles may be influenced to assume a uniform lateral orientation. This was achieved by choosing the geometrical dimensions of the hydrodynamic focusing flow path. High speed photographs of fixed chicken erythrocytes oriented in experimental chambers are presented.

The optical flow-through instruments commonly used illuminate cells radially with a laser beam. Gledhill *et al.* (1) have shown that the deoxyribonucleic acid distribution curves of different sperm cell types consist of a peak with an extension to higher fluorescence values, when measurements with the fluorescence excited by a radially incident laser beam are performed. Based on a number of observations, they conclude that the shape of the distribution curves is disturbed, probably due to the fact that the flat sperm cells are oriented in a random way in the optical sensing zone of the flow system. Sperm cells did show deoxyribonucleic acid distribution curves without extension to the right (8) when measured in our axially illuminating "Fluvo-Metricell" (5) instrument. If, however, radial laser illumination is used, disturbances in the orientation are best avoided by orienting all cells uniformly. In the following, we describe a simple method to uniformly orient flat cells by flow forces.

REGULAR OCCURRENCES IN A LAMINAR HYDRODYNAMIC FOCUSING FLOW PATH

In a focusing flow system, the fluid flow is determined by the pressure change in the flow from wider cross-sections of the flow path to that in its smaller cross-sections (Fig. 1). Because the walls of the constricting flow path, *e.g.*, the walls of a tapered tube, exert concentrating and accelerating forces on the fluid, it needs higher velocities (the principle of mass conservation) to pass through smaller cross-sections. The accelerating forces are exerting their effect in the axial direction, whereas the transaxial direction is determined by constricting forces. In the following the constricting forces will be treated as two equal but counteracting forces, f_h and f_v. The quantity of these two concentrating forces depends on the degree of constriction of the flow path in the horizontal or the vertical direction, respectively. We define the degree of constriction by the ratios A/a and B/b, of the horizontal or vertical dimension of the flow path at the particle inlets (the outlet of the particle injection tube) A and B to the respective horizontal or vertical dimension at the end of the constricting paths a and b. In the following, we discuss the movement of particles along the line of symmetry of revolution (flow axis). If the symmetry of revolution does not exist, we consider those particles that move along the intersection line of the vertical and horizontal planes of symmetry leading through the flow path. Thus, the particles can be considered as moving along a straight line through the flow system, and additional side forces that may act outside the planes of symmetry can be neglected. Three different types of flow paths which influence the moving particles will be discussed.

FLOW CONSTRICTION NOT AFFECTING THE LATERAL ORIENTATION

Figure 2 shows cross-sections of flow paths, whereby A/a = B/b, *i.e.*, the cross-sections along the flow path, are transformed according to the law of similarity. If we neglect the friction of the tube walls (potential flow), every partial area of the cross-section A × B may be fitted into the reduced but similar cross-section a × b. The dimensions of the cross-sections in flow will be smaller, but the shape itself will stay similar; *i.e.*, a partial square field F somewhere in the cross-section A × B appears in the cross-section a × b also as a square field F′, but reduced by the factor a/A or b/B (both are equal in this case; see Fig. 2, right panel). Similar reduction of the laminar flow areas implies that no preferential side force is affecting the flow or the particles moving in the flow. These particles enter the flow in a random orientation. The constricting or focusing forces try to compress them laterally and orient them with their longest extension in the main flow direction as previously described in (7). However, the *a priori* lateral orientation remains unchanged.

FLOW CONSTRICTION CAUSING VERTICAL ORIENTATION

Let us assume a change in the geometrical dimensions of the constricting flow path, so that similarity no longer exists between the cross-sections A × B and a × b (Fig. 3A). A/a > B/b implies $f_h > f_v$. A small square field in the flow cross-section A × B no longer appears as a reduced square field in the cross-section a × b.

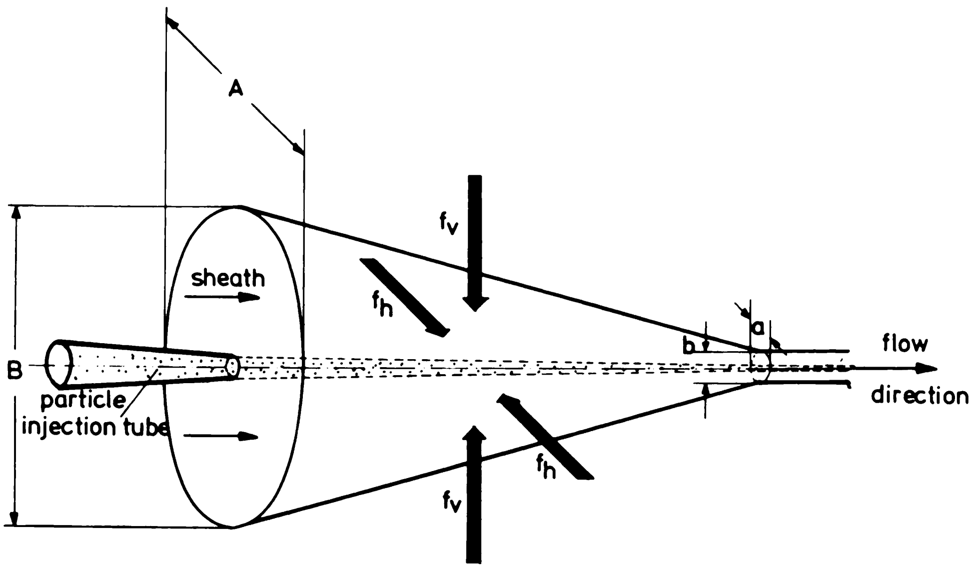

FIG. 1. Dimensions and forces exerted in an usual focusing flow path with symmetry of revolution. f_h, constricting flow forces that have a horizontal effect; f_v, flow forces acting vertically. A, horizontal dimension of the flow path at the particle inlet; *a*, horizontal dimension of the orifice or nozzle; *B*, vertical dimension of the flow path at the particle inlet; *b*, vertical dimension of the orifice or nozzle.

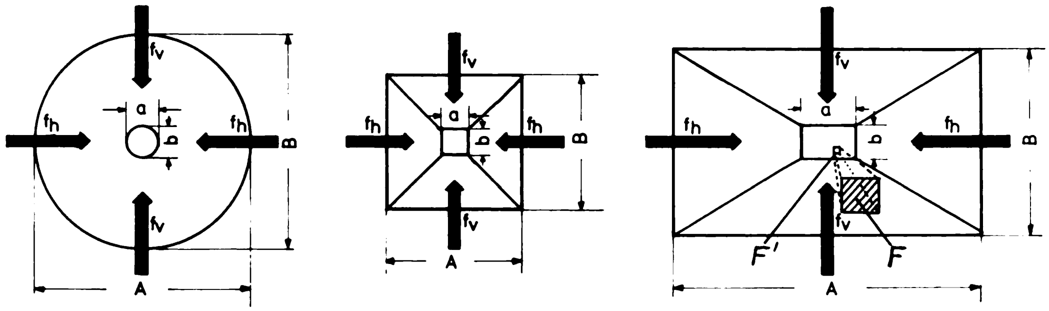

FIG. 2. Examples of the cross-sections of the flow path, if the condition A/a = B/b is fulfilled. By the law of similarity no preferential side forces influence the flow and the particles in flow. Every area in the cross-section A × B of the flow, e.g., the square field F, is found as a equally shaped field in the cross-section a × b, but scaled down (square field F′). For explanation of lettering see Figure 1.

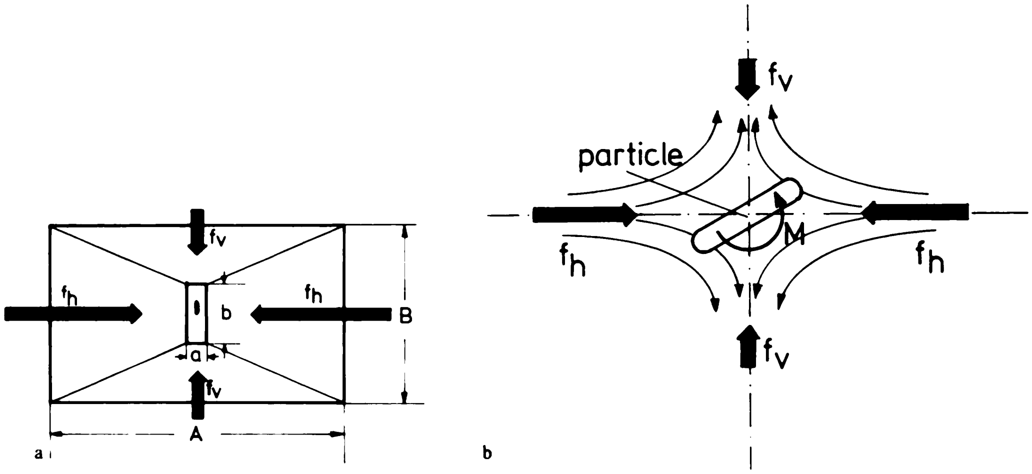

FIG. 3. A, cross-sections of a flow path where A/a > B/b. For lettering see Figure 1. The dissimilar transformation of the cross-sections causes preferential horizontal side forces which generate additional up and down flow components. These components are responsible for the vertical orientation of flat cells. B, detailed conditions of the flow around a particle. The main flow direction is normal to the plane of the paper. The decreased vertical force f_v enables a superpositioned flow in an up and down direction at both sides of the vertical plane of symmetry. These flow components are indicated by the thin arrows. The torque M produced by them rotates flat cells to the stable vertical orientation.

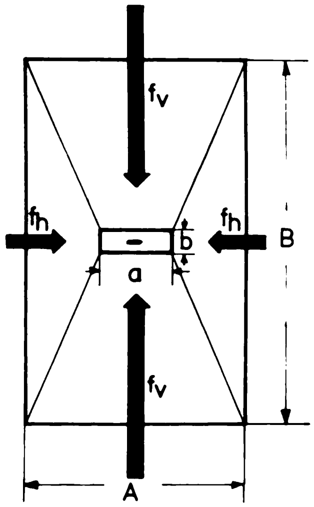

FIG. 4. Cross-sections of a flow path where A/a < B/b. The conditions are inverted compared to those described in Figure 3. Flat cells are horizontally oriented.

It is transformed to a rectangular field with its longer extent in the vertical direction. The flow itself no longer follows the law of similarity. By reasons of mass conservation an additional transformation of the laminar flow takes place. One part of the fluid at the right and left sides of the cross-section A × B has to be transported by the flow to the upper and lower areas of the constricted cross-section a × b. This additional up and down flow, caused by the increased horizontal constriction of the flow path, orients flat cells in a vertical position (Fig. 3B). Cells that initially were in a horizontal orientation are now in an unstable position. Small distortions initiate the change of the orientation from the unstable horizontal to the stable vertical position. In this stable position the horizontal forces f_h are normal to the flat sides of the particles.

FLOW FORCES CAUSING HORIZONTAL ORIENTATION

If the dimensions of the flow constriction are chosen such that A/a < B/b, then $f_h < f_v$. Under these conditions the increased vertical flow compression causes horizontal orientation of flat particles. The mechanism is the same as that described above.(Fig. 4).

DEMONSTRATION OF THE ORIENTATION EFFECT

Figure 5 shows schematic diagrams and Figure 6 shows authentic photographs of the two plexiglass chambers that we constructed to demonstrate the uniform orientation of flat particles. Both chambers were mounted on a Zeiss

Universal microscope. To photograph the moving cells, a short time illuminating device was used, as previously described (2, 3). The particle flow was controlled by the regulating device of our Metricell instrument (4). Because fixed chicken erythrocytes are flat, we used them as test particles. Figure 7 depicts these cells suspended in distilled water while passing through the orifices of both chambers. The sheath fluid also consisted of distilled water. The applied suction was about 0.2 atmosphere. Because the cover slip of the chambers interferes with the horizontal planes of symmetry, the straight particle path at the intersection line between both symmetry planes could not be used in this experiment. Therefore, the particles moved in the vertical planes of the symmetry and slightly below its horizontal planes of symmetry. As the results indicate, this fact does not influence the orientation effect strongly.

DISCUSSION

Cells that are horizontally oriented do show an increased horizontal spread. This clearly is explained by the decrease of the hydrodynamic focusing in the horizontal direction in that case. To obtain a reasonable spread into the direction that focuses, to a lesser degree, the outlet width of the particle tube, leading into this direction should be as small as possible. Another possibility to induce a reasonable cell spread could be a second focusing stage without lateral orientation. Some of the horizontally oriented cells are in their longer extension not oriented along the flow axis. The small angle of 12° in the horizontal constriction and rough walls

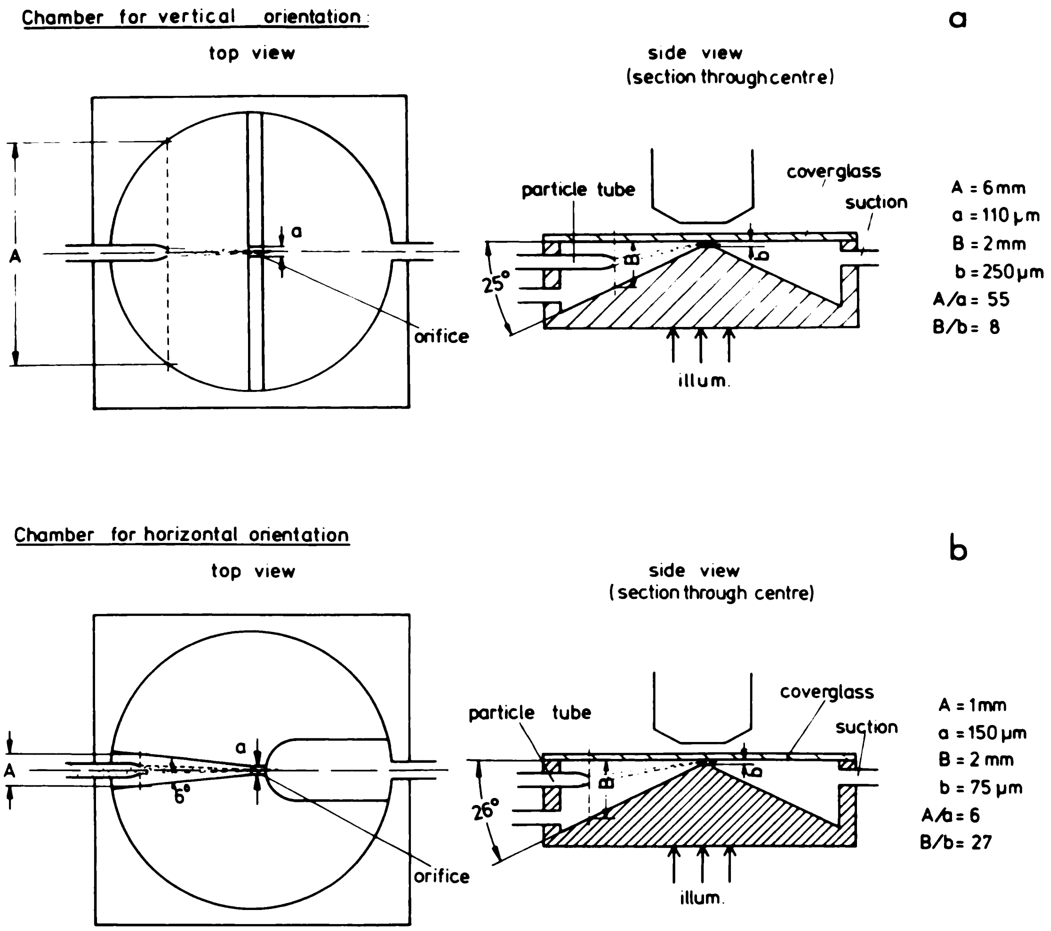

FIG. 5. Schematic diagrams of the two plexiglass chambers used to demonstrate the lateral orientation of flat cells.

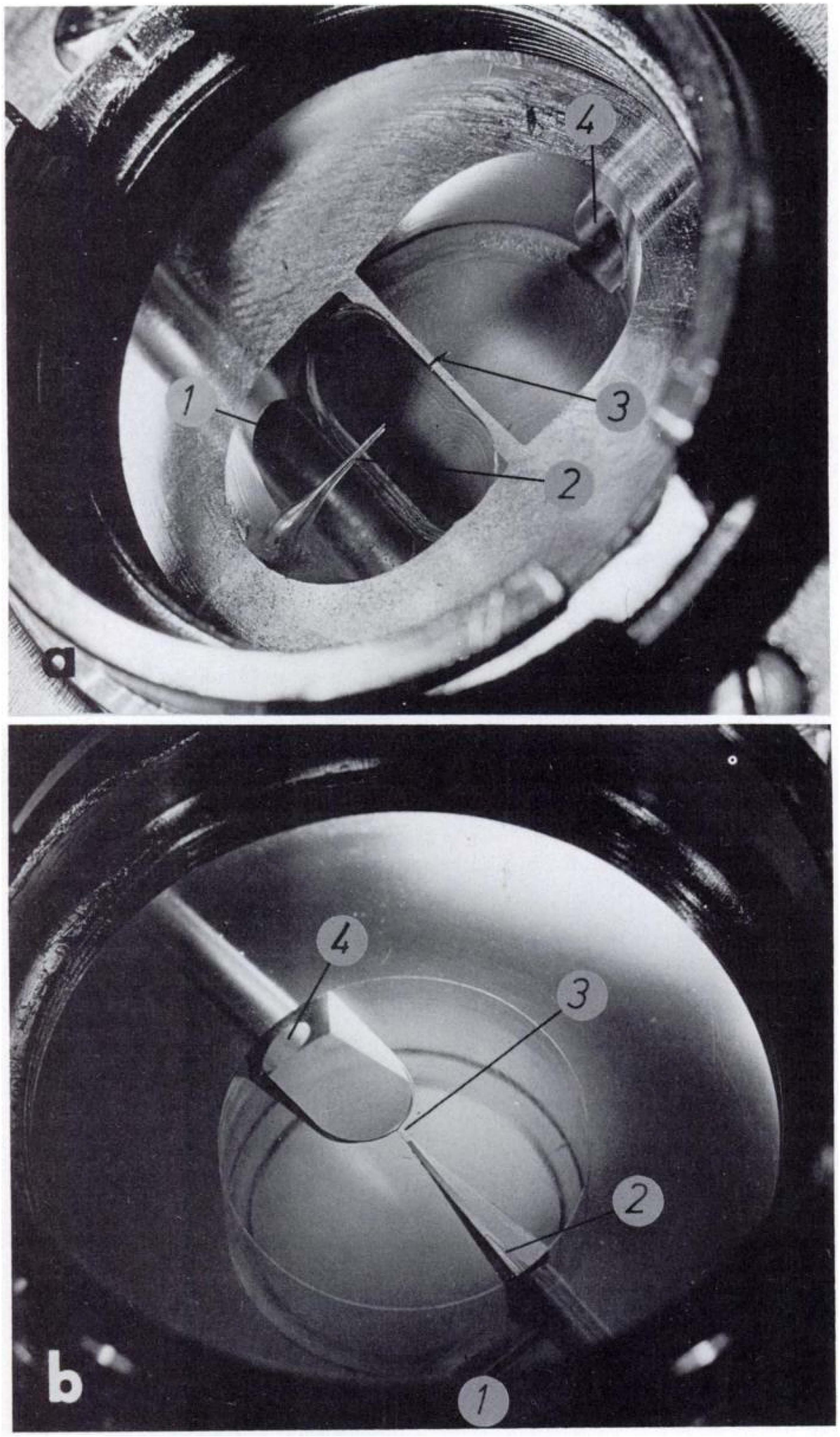

FIG. 6. Photographs of the flow paths of both experimental chambers. A, vertically orienting chamber; B, horizontally orienting chamber. 1, sheath fluid inlet; 2, particle tube; 3, orifice; 4, suck connection.

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