SIMPLE METHOD OF RECORDING RELATIVE VOLUME CHANGES IN CELLS ADHERENT TO GLASS

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Measurement of the volumes of living cells can yield important information on the mechanisms regulating their water and electrolyte balance. Methods of measuring cell volumes currently available have disadvantages which restrict their wide application. They are either extremely laborious, such as, for example, the method of measuring the diameter of the cells visually with the aid of an ocular micrometer [1] or they require the use of complex electronic, computer, or laser techniques. Such methods include the Coulter ZM-Channelizer (Coulter Electronics) conductometric method [2, 3], which is most frequently used, the method of measuring the volume of cells by a system consisting of microscope, television equipment, and computer [4], and finally, the method of measuring volumes based on low-angle scatter of light [5].

The method suggested in this paper does not require the use of complicated and expensive electronic equipment, it is simple and convenient in use, and with some degree of modernization, it can be used for extensive clinical investigations. It is based on the principle of determination of the concentration of particles by means of a microscope with dark-field condenser, known in colloid chemistry [6]. Development of the method was preceded by experimental observation of the fact that the luminance of cells is increased in the dark field of the microscope after they have shrunk in hypertonic solutions, whereas the luminance of cells is reduced if they swell in hypotonic solutions. In our view changes in luminance of cells in anisotonic solutions are basically connected with a change in the scatter of light on the ultramicroscopic membrane structures of the cell.

To put the method into practice, conditions had to be determined under which the number of cells in the field of vision of the microscope would remain constant throughout the duration of the experiment. This constancy was maintained by adhesion of the cells to the glass of the experimental chamber. The luminance of the cells was recorded by means of a microscope (MM-1) with dark-field condenser and with photoelectric multiplier (FÉU-1) built into the microscope, a UPT (U7-2) dc amplifier, and KSU-4 automatic recorder. To obtain a qualitative image of the cells in the dark field, a powerful light source (90-500 W) with stabilized supply voltage must be used. A diagram of the system is shown in Fig. 1.

A light source with stabilized voltage generates a constant light flux. The microscope with dark-field condenser picks out that part of the light flux which is scattered by the cells. By means of a photoelectric cell, dc amplifier, and automatic recorder, the light flux picked out by the microscope is automatically recorded. The design of the experimental chamber allows replacement of the solutions bathing the cells.

The chamber consists of a transparent plastic plate 1.5-2 mm thick with a hole 10-15 mm in diameter at its center; coverslips are placed over the hole in the plate at either side and are fixed with melted paraffin wax. To allow liquid to flow through the chamber, a hole 0.8-1 mm in diameter is drilled in the plate, parallel to its flat surface, and passing through the center of the central compartment. To fill the chamber with cell suspension, thin soft polyethylene tubes each 20-25 cm long are introduced into the holes in the chamber. The end of one tube is dipped into the cell suspension and a negative pressure is applied to the end

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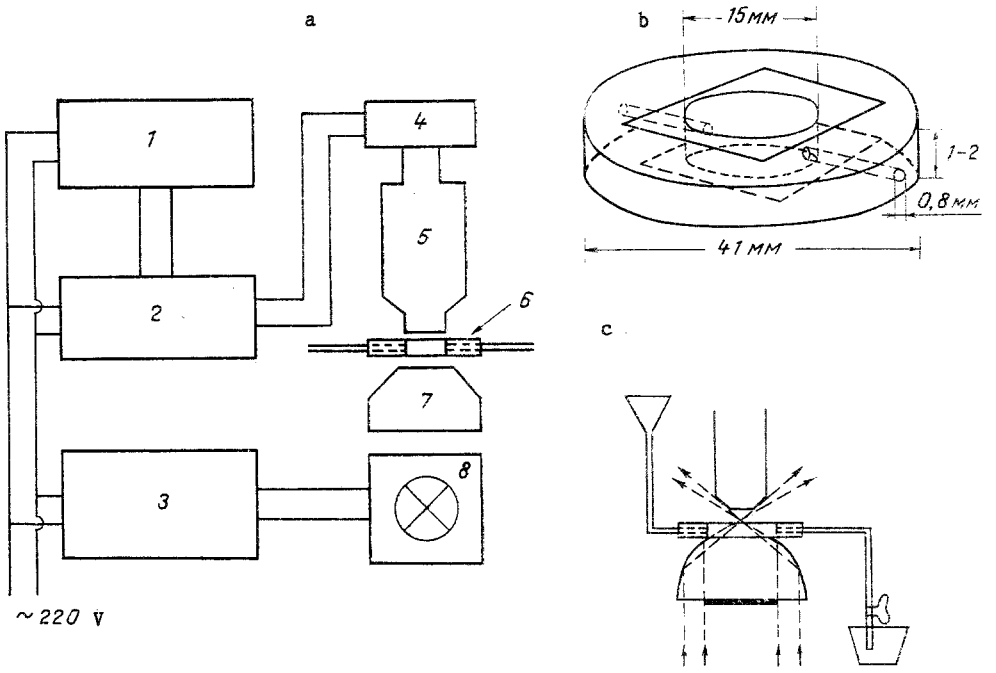

Fig. 1. Diagram of apparatus. a) Block diagram, b) experimental chamber, c) course of light rays in dark-field condenser; 1) automatic recorder; 2) dc amplifier; 3) stabilizer; 4) photoelectric cell; 5) microscope; 6) continuous-flow chamber; 7) dark-field condenser; 8) light source.

of the other tube by means of a compressible bulb, until both tubes and the chamber are completely filled with suspension without any air bubbles. The ends of the tubes are then connected together with a short connecting tube, the chamber is placed with its front side downward, and the cells are allowed to settle on the glass. Usually from 10 to 30 min is required for adhesion of the cells to the glass. After the cells have adhered to the glass the chamber is moved to the stage of the microscope so that its top side faces the objective of the microscope (magnification of objectives 10 × and 20 ×), the light source, amplifier, and recorder are switched on, and the image having been obtained, the density of settling of the cells on the coverslip is tested. The image observed in the dark-field microscope consists of brightly luminescent cells against a black background. The optimal density of settling of the cells is considered to be that at which the whole field is studded with cells, with only small areas of empty space between them; with this density of settling the amplitude of the cell responses to the external agent is maximal. After the image has been obtained, the chamber must be irrigated for 2-3 min with isotonic solution in order to wash off the cells which are not adherent to glass. To do this, the tubes must be disconnected and one end connected to a funnel, whereas a clamp is applied to the other tube and its end is lowered into the vessel to collect the fluid flowing out. The funnel is filled with isotonic solution and placed above the level of the microscope stage, to allow the fluid to enter the chamber under low hydrostatic pressure. The rate of flow must be one or two drops per second. Next the initial luminance of the cells in isotonic solution is recorded. For this purpose, a short-term trace of the initial levels is obtained on the automatic writer with the photoelectric cell covered (dark-field L), and when the path of the rays in the microscope is switched to the photoelectric cell (isotonic L). It is essential to record the initial luminance of the cells (L_{in} = $L_{isoton} - L_{dark\ field}$) in order to compare quantitatively the results obtained in different experiments, for it is impossible to achieve identical density of settling of the cells in these experiments. Knowing the initial luminance of the cells it is easy to find the relative luminance. Relative luminance (L_{rel}) is taken to be the ratio of luminance of the cells in response to the agent ($L_{effect} - L_{isoton}$) and the initial luminance (L_{in}):

$$L_{rel} = \frac{L_{effect}(N) - L_{isoton}(N)}{L_{isoton}(N) - L_{dark\ field}},$$

where N is the number of cells in a field of vision of the microscope (Fig. 2). The value of L_{rel} is independent of the number of cells in the experiment and, we can postulate, reflects

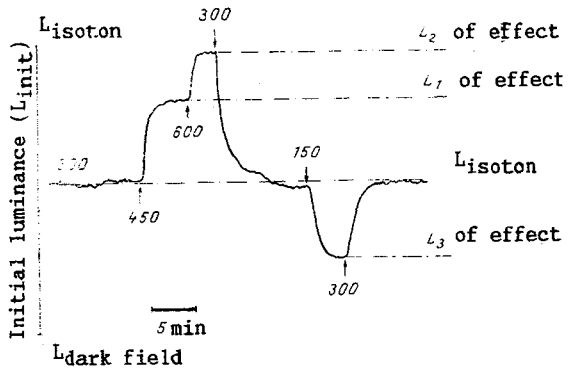

Fig. 2

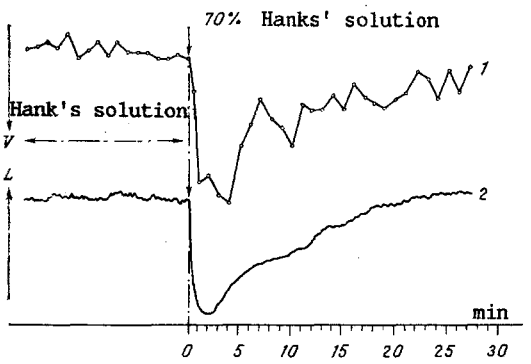

Fig. 3

Fig. 2. Changes in luminance of mouse peritoneal macrophages reflecting change in cell volume in NaCl solutions of different osmolarity (300, 450, 600, 300, 150, 300 milliosmoles/liter).

Fig. 3. Change in volume (V) and luminance (L) of mouse peritoneal macrophages during long-term exposure to hypotonic solution (70% Hanks' solution), recorded by a "Magiscan-2" complex (1) and by the suggested method (2).

the relative change in cell volume, i.e., $L_{rel} = \frac{\Delta L}{L} = k \frac{\Delta V}{V}$, where $\frac{\Delta V}{V}$ denotes the relative change in cell volume and k is a coefficient of proportionality.

To test this hypothesis parallel investigations were made of the response of the cells to a change in osmolarity of the medium by the method described above and by a morphometric method using the "Magiscan-2" combined measuring and computer system (Joyce–Loebl). The system includes a microscope, a television camera, and a computer. The standard software of the complex enables the area of each cell to be distinguished and the data presented in a form convenient for the experimenter. The results of these investigations are given in Fig. 3 and they show that our suggested method does in fact record changes in cell volume.

To demonstrate the possibilities of the method, the curves in Fig. 2 reflect changes in volume of the cells during repeated and rapid change of the solution surrounding the cells. The character of response of normal viable cells to anisotonia depends on the duration of their stay in the anisotonic solution: with short exposure to the anisotonic solutions the cells behave like passive osmometers (Fig. 2), but by contrast, during long-term exposure to anisotonic solutions regulatory changes of volume are observed in the majority of types of cells immediately after the passive phase (Fig. 3), connected with activation of additional ion transport systems in the cells and the redistribution of water.

We have tested the method on different types of cells (human blood neutrophils, mouse peritoneal macrophages, Ehrlich's ascites carcinoma cells, fibroblasts) and in all cases changes in osmolarity of the medium were well recorded. Volume changes of maximal amplitude were recorded in the case of unactivated cells, spread out only a little or not at all on the glass.

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