METHOD OF DETERMINING ACTIVITY OF CELL SUSPENSIONS DUE TO THEIR INTRINSIC MOTILITY

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There are two known methods of evaluating the activity of a cell suspension due to intrinsic motility of the cells. The most widely used method is visual evaluation under the microscope [3]. In this method the fraction of cells moving in a straight line is determined [4]. Sometimes, for example when activity of suspensions of spermatozoa is evaluated, activity is expressed in points of a 10–point scale. Visual analysis is qualitative and subjective in character. Moreover, when activity is evaluated in this way the velocity of rectilinear cell movement is disregarded. The second method of evaluating activity of cell suspension with intrinsic motility is to measure the mean velocity of rectilinear movement of the cells by optical displacement spectroscopy [1]. In this case, the measure of activity is taken to be the mean velocity of rectilinear movement of the cells, and it is evaluated objectively. A drawback of this method is that the parameter of activity adopted disregards the proportion of motile cells.

This paper describes a study of the possibility of using an instrumental method of evaluating activity of cell suspensions due to their intrinsic motility, and taking account both of the fraction of motile cells moving in a straight line and also their average velocity.

EXPERIMENTAL METHOD

Activity of a cell suspension due to intrinsic motility of the cells can be described by a parameter A, which takes into account both the fraction of motile cells and their average velocity, determined by the equation

$$A = a\frac{c_m}{c}v_m$$

where a is a coefficient of proportionality, c_m the concentration of motile cells, c the total concentration of motile (c_m) and nonmotile (c_{nm}) cells, and v_m the average velocity of the motile cells.

The value of A can be determined by simultaneously measuring the frequency f with which the cells pass through a given region of space, and the integral intensity I of scattering of light by the cell suspension. It can be shown that if in the space within which the cells move a region with diameter d is distinguished, the frequency of entry of cells into this region will be proportional to the concentration of motile cells and their average velocity

$$f = a_1 c_m v_m, \tag{1}$$

where $a_1 = 2\pi^2 d^2$.

The integral intensity of scattering of light is proportional to the total cell concentration

$$I = a_2 c = a_2 (c_m + c_{nm}), \tag{2}$$

where a_2 is a constant coefficient.

Equations (1) and (2) are valid for a homogeneous suspension with low concentration c. Thus

$$A = f/I.$$

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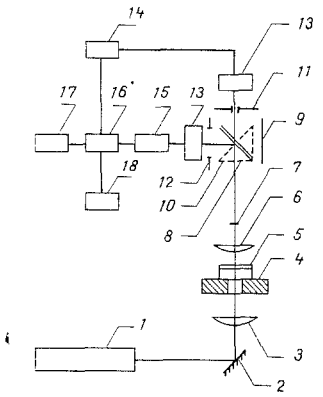

Fig. 1.

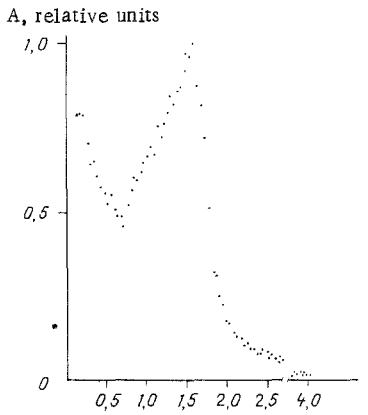

Fig. 2.

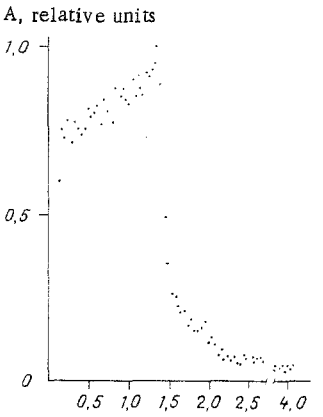

Fig. 3.

Fig. 1. Block diagram of apparatus. Explanation in text.

Fig. 2. Dependence of activity (A) of suspension of bovine spermatozoa on time. Diluent – 0.9% sodium chloride. Abscissa, time (in h).

Fig. 3. Dependence of activity (A) of suspension of bovine spermatozoa on time. Diluent – 2.9% sodium citrate. Abscissa, time (in h).

To determine the value of A experimentally an apparatus, a block diagram of which is shown in Fig. 1, was constructed. Radiation of a helium-neon laser 1 is reflected by a vertical mirror 2, focused by the lens 3 into a cuvette containing the sample 5, placed on a constant-temperature stage 4. The micro-objective 6 and spatial filter 7 form an image of the cells in the planes of the diaphragms 11 and 12. Diaphragms 11 and 12 are equal in diameter to the mean size of the image of the cell and about 100 times greater than the mean size of the cell respectively. By placing the prism 10 at the site of the light-dividing plate 8, the image of the cells can be observed visually on the mat screen 9. Photodiodes 13 transform the photic signal into an electrical signal. Block 14 includes an amplifier, discriminator-shaper, and integrating counter and serves to count the number of pulses evoked by passage of the images of the cells across the field of the diaphragm 11. Block 15 includes an amplifier and a voltage to frequency converter and measures the integral intensity of scatter. Block 16 controls and calculates the value of A, assigns the measurement time of 60 sec, performs division, and displays the result on the digital printer 17 and the decimal indicator 18. Trials of the method and apparatus were carried out on a suspension of bovine spermatozoa, frozen in granules to –196°C. The granules were thawed at (40 ± 1)°C. The diluting agents were a 0.9% solution of sodium chloride and a 2.9% solution of sodium citrate. The power of the laser radiation used was such as not to cause changes in activity of the spermatozoa.

EXPERIMENTAL RESULTS

The results of measurements of activity of suspensions of bovine spermatozoa, demonstrating the scope of the method, are given in Figs. 2 and 3. By means of the method, a number of characteristic features of the behavior of suspensions of spermatozoa with time can be demonstrated.

Figure 2 shows that dependence of activity of the suspension of spermatozoa in 0.9% sodium chloride on time was characterized by a peak, observed soon after thawing (beginning of counting), followed by a sharp decline of activity.

This type of curve can be explained by the specific nature of the biological activity of suspensions of spermatozoa, leading to changes in pH of the medium. Motile cells secrete lactic acid into the medium, and its accumulation leads to a gradual diminution of activity [2]. Meanwhile decomposition of dead cells causes the release of substances with an alkaline reaction into the medium, neutralizing the lactic acid. At the moment of neutralization an increase in activity can take place both through an increase in the velocity of movement (v_m) and on account of concentration of motile spermatozoa c_m, for not all nonmotile spermatozoa are dead. The sharp decline of activity immediately after the peak is due to the fact that with an increase in activity, secretion of lactic acid also increases. At a certain moment it reaches a level such that most spermatozoa cease to move and die. Only a small proportion preserve their motility for some time, and this is responsible for the "tail" on the curve.

The use of sodium citrate as diluent (Fig. 3) changes the character of dependence of activity of the suspension on time. Since sodium citrate is a buffer, keeping the pH of the medium constant [2], the action of lactic acid is weakened. As a result of this, activity rises slightly for some time, then falls with a longer "tail".

The suggested method and the apparatus used for it enable activity of a cell suspension due to intrinsic motility of the cells to be estimated, allowing for the relative proportion of motile cells and their average velocity. The simplicity of the technical solution means that the apparatus as designed can be used for automated express monitoring of cell motility in the diagnosis of human infertility and for quality control of the sperm of livestock used for artificial insemination and of bacteria used in the manufacture of fertilizers.

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FLOATING CULTURES OF FETAL PANCREATIC CELLS

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Cultures of pancreatic islet cells (IC) from human and mammalian fetuses are nowadays widely used in experimental endocrinology and transplantology. Such cultures have begun to be used also in recent years for clinical transplantation into diabetic patients [3, 7, 8, 10, 11, 15]. For this reason, the development of reliable methods of obtaining insulin-producing cultures from the fetal pancreas of man and animals has assumed great importance. Previously [1, 6] the writers described a method of obtaining monolayer focal cultures of IC by enzyme treatment of material with collalytin and trypsin combined with microdissection and differential sedimentation. Monolayer cultures prepared in this way contained many B cells, actively secreting insulin [4, 5]. Cultures of this kind have been used for xenografting into rats with alloxan diabetes [2, 9]. However, a drawback of this method was the loss of large numbers of IC during multistage processing of the material, especially when tissue microfragments were being processed on the magnetic mixer and during centrifugation.

The aim of this investigation was to develop a new, simpler, and more rational method, not using centrifugation to be free from the disadvantage described above, capable of yielding cultures of IC in sufficient numbers for producing a therapeutic effect on clinical transplantation.

EXPERIMENTAL METHOD

The cadaveric pancreas from human fetuses at the 16th–25th week of intrauterine development (abortions, lower-segment caesarian section) and from pig fetuses at 2.5–3 months of intrauterine life were used as the source of the cell cultures. The pancreas was removed under sterile conditions, washed thoroughly in Hanks' solution with antibiotics, and freed from capsule and large connective-tissue bands containing blood vessels. The pancreas was then cut into fragments measuring 2–3 mm and immersed in 0.2% collalytin solution in Hanks'

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