EFFECT OF CONDITIONS OF CULTURE ON QUANTITATIVE ASSESSMENT OF VIABILITY OF LIVING TISSUES

V. P. Pashchenko

UDC 57.085.23

The viability of tissue explants of mouse kidneys, estimated quantitatively by growth in plasma-free culture, was shown to depend on several factors connected with the conditions of culture. The effect of methods of treatment of the serum, pH of the medium, and the character of distribution of the tissue explant during culture, the size of the explants, and the firmness of attachment of the tissue fragments to the substrate on this index was demonstrated.

KEY WORDS: *tissue culture; viability; conditions of culture.*

The tissue culture method, when used to assess the viability of living cells and tissues, is more sensitive than many other methods [7] and, for that reason, it is often used in oncology, pharmacology, transplantology, and other branches of medicine [2, 3, 9, 10]. However, no comparative quantitative assessment of the effect of various conditions of culture on the viability of tissue explants has yet been undertaken, and this makes it difficult to develop standard procedures for estimation of this index [4, 6, 8].

It was therefore decided to undertake a quantitative study of the viability of tissue explants of animal kidneys cultured under different conditions, by using the method of plasma-free tissue culture for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on mice weighing 18-25 g. Pieces of tissue from the kidneys and other organs measuring 0.3-0.6 mm^2 were cultured in tubes on the surface of coverslips, separated by a distance of 4-6 cm from the bottom of the tubes. Plasma-free culture was carried out at 37°C in medium No. 199 with 10% bovine serum and antibiotics (100 units/ml each of penicillin and streptomycin). The bovine serum was inactivated at 56°C for 30 min.

After explantation of the tissue fragments the tubes were closed with stoppers and racked in a vertical position in an incubator at 37°C to promote better attachment of the tissue fragments to the glass. This step of the culture procedure was immediately followed by placing the tubes in permanent culture racks, in which they were inclined at an angle of 2.5-3°.

To assess growth of the cultures, between 40 and 60 tissue explants distributed in 10 tubes, each containing 4 or 6 fragments, were used. The viability of the tissue was estimated from the size of the cell colony growing around the central explant during culture for 72 h. The number of cells was counted directly in all colonies by means of an ocular grid [1]. The degree of attachment of the tissue fragments to the surface of the substrate (coverslip) was judged from the number of tissue fragments remaining on the glass after culture for 72 h. The results were subjected to statistical analysis by a nonparametric method using Wilcoxon's criterion and Spearmen's rank correlation coefficients [5].

EXPERIMENTAL RESULTS

The observations showed that the estimation of viability of tissue explants by the culture method is influenced by several factors connected with the conditions of culture.

Problem Laboratory, Arkhangel'sk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 629-631, November, 1978. Original article submitted April 14, 1978.

 0007-4888/78/8611-1554$07.50

TABLE 1. Importance of Quantity of Serum, Size of Explants, and pH of Medium for Quantitative Assessment of Viability of Kidney Tissue by the Plasma-Free Tissue Culture Method

Factor affecting growth of cultures and attachment of explants to coverslip	Index	Numerical values of indices
Importance of quantity of serum in medium	Concentration of serum in medium, %	5 10 20 30 40
	Number of explants per coverslip	20.7 26.8 32.8 44.1 46.1
	P	<0.05 <0.01 <0.01 >0.05
	Size of cell colony	5,8 15.8 26.7 31.8 28.1
	P	<0.01 <0.01 >0.05 >0.05
Effect of length of exposure of tubes with cultures before their distribution in racks for culture	Exposure, min	0 30 60 90 120
	Number of explants per coverslip	25.2 34.4 36.1 36.7 36.0
	P	<0.01 >0.05 >0.05 >0.05
	Size of cell colony	25.2 39.2 31.3 25.0 17.1
	P	<0.01 <0.01 >0.05 <0.05
Importance of pH of medium	pH of medium	6,4 6.8 7.1 7.3 7,5 7,8
	Size of cell colony	26 34 38 34 36 35
	P	>0.05 <0.01 <0.05 >0.05 >0.0
	Size of cell colony	4,3 42,4 54,4 52,1 27,8 10,9
	P	0,01 0,01 0,05 0,01 0,01
Role of size of tissue explants	Size of tissue explant, mm	0.1 0.26 0.50 0.82 1.35
	Size of cell colony	36.6 26.2 22.8 20.5 15,3
	P	>0.05 >0.05 >0.05 >0.05

TABLE 2. Effect of Inactivation and Keeping of Bovine Serum Used for Culture and also of Certain Conditions of Explantation on Assessment of Viability of Kidney Tissue by a Culture Method

1) Factor influencing culture growth	I	II	P	%
Effect of uninactivated (I) and inactivated (II) serum	41,8	24,0	$<0,01$	—42,6
Use of serum kept for 9 months at temperatures of −10 to −15°C (I) and from +2 to +4°C (II)	27,1	16,1	$<0,01$	—40,6
Effect of properties of glass from which tubes in use for 1 year (I) and new tubes (II) were made	19,6	13,7	$<0,05$	—30,3
Importance of distance between cultures: 1,5 mm (I) and 4 mm (II)	77,9	60,8	$<0,05$	—22,0
Distance from explants to surface of medium: at boundary of medium (I), at distance of 1 mm (II)	50,8	21,6	$<0,01$	—57,5
Effect of removal of explant from surface of coverslip after culture for 72 h on subsequent growth of tissue cultures during 24 h: without removal of explant (I), with removal of explant (II)	93,6	46,4	$<0,01$	—50,5

The results given in Table 1 are evidence that plasma-free tissue cultures have several features in this respect distinguishing them from other methods of culture and due, in particular, to the unequal degree of attachment of the primary tissue explants to the surface of the solid substrate.

Significant positive correlation was found (Table 1) between the concentration of serum in the medium, the degree of attachment of the kidney tissue explants to the coverslip, and the size of the growing cell colonies ($P < 0.05$). Further investigations of this factor showed that the type of tissue of the explant was an important factor influencing attachment of the tissue explants to the surface of the coverslip: Fragments of tissue from the lungs, testes, intestine, and brain adhered to the surface of the glass less firmly than pieces of tissue from the liver, kidneys, and spleen. The donor's age also played a role: Fragments of kidneys from adult animals fixed to the coverslip more firmly than fragments from young animals. The origin of the serum also had some effect: Rabbit and human serum had less effect on adhesion of the tissue fragments to the glass than bovine serum. The size of the explant also was important: Under otherwise identical conditions smaller tissue fragments adhered to the surface of the coverslip better than large fragments. This last fact is evidently the reason for the significant negative correlation between the size of the tissue explant and growth of the cultures ($P < 0.05$).

The effect of various factors connected with treatment of the serum and the method of arrangement of the cultures on quantitative assessment of viability of kidney tissue is shown in Table 2. The most important of these factors are the method of treatment and the keeping time of the serum and also the position of the explant in culture relative to the

surface of the medium. Usually the most rapid growth was found in the case of cultures placed at the boundary between the liquid and gaseous phase of the medium. Dependence of growth of the cells from the central tissue fragments on to the surface of the glass and the liberation of products of tissue metabolism stimulating cell division from it [4, 11].

These investigations thus showed that various factors connected with the conditions of culture affect quantitative assessment of the viability of the tissue in culture to different degrees. This fact must be taken into account both when cultures are set up for quantitative investigations and also when methods of assessing the viability of living tissue explants are developed.

LITERATURE CITED

1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973).
2. M. S. Benyumovich, Usp. Sovrem. Biol., 75, No. 2, 314 (1973).
3. Yu. E. Blok and A. S. Loginov, Byull. Éxp. Biol. Med., No. 5, 76 (1969).
4. V. I. Gavrilov, Transplantable Cells in Virology [in Russian], Moscow (1964).
5. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medical and Biological Research [in Russian], Leningrad (1973).
6. M. I. Levi, Tissue Culture in the Study of Poliomyelitis [in Russian], Stavropol' (1951)
7. Yu. M. Lopukhin and É. M. Kogan, Criteria of Viability of Organs and Tissues before Transplantation [in Russian], Moscow (1975).
8. J. Paul, Cell and Tissues in Culture, Longman, New York (1975).
9. I. M. Surikov, Tsitologiya, No. 7, 83 (1965).
10. E. A. Timofeevskaya, Vest. Akad. Med. Nauk SSSR, No. 9, 70 (1966).
11. N. G. Khlopin, Tissue Culture [in Russian], Moscow (1940).

ORIENTATION OF NONSPHERICAL CELLS IN BLOOD FLOWING THROUGH A VESSEL

E. G. Popov

UDC 612.13

Photometric experiments on model blood vessels showed that nonspherical blood cells are oriented in the flow with their short axes along the radius of the vessel.

KEY WORDS: *blood cells; physiological function.*

During investigation of the hemodynamic properties of blood the determination of the orientation of blood cells without spherical symmetry frequently arises; it is important for the study of their kinematics and, possibly, their physiological functions.

Since the available data are insufficient to solve this problem, the investigation described below was carried out to determine the orientation of nonspherical cells in the blood flow by recording changes in optical density of a cell suspension in plasma moving through cylindrical cuvettes with cross sections of different shapes, and also in a resting state.

EXPERIMENTAL METHOD

The optical density of the suspension was measured as it passed through a glass cuvette with rectangular cross section of 0.7 × 14 mm and through a tube with a bore of 2.5 mm. An LG-56 helium-neon laser with wavelength of 632.8 nm, the beam of which passed perpendicularly to the flow, was used as the source of light. The cuvette with the rectangular cross section was illuminated along the larger side, the tube through its central part. Changes in optical density were observed by means of a square detector, the signals from which were recorded on an automatic writer.

All-Union Cardiological Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR E. I. Chazov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 631-632, November, 1978. Original article submitted May 19, 1978.

 0007-4888/78/8611-1556$07.50