Quantitative Microtomography of the Early Mammalian Embryo by Laser Scanning Microscopy

A. G. Pogorelov and V. N. Pogorelova

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The blastomer volume was measured by three-dimensional (3D) reconstruction of a series of successive optical sections of the early mouse embryo. Changes in cell volume during osmotic shock were studied. Incubation of a two-cell embryo in Dulbecco's medium induced slow shrinkage of the embryonic cells followed by recovery of its initial volume. A regulatory phase of osmotic shock compensation is characteristic of a blastomer under hypotonic conditions.

Key Words: *early mouse embryo; osmotic shock; blastomer volume; laser scanning microscopy; 3D reconstruction*

Changes in cell volume induce a cascade of transformations at the molecular genetic level: protein synthesis [1], gene expression [2,9], cell death [4], partenogenesis, activation of ionic channels [6]. Activity of MAPK p38 protein and CCM2 volume increase in the cells cultured under hyperosmotic conditions [7]. The *in vitro* cell systems (cell culture or isolated early embryo) are particularly sensitive to osmotic shock. Even a short exposure to hypertonic medium causes disorders in the embryonic development [3,10]. Hence, the conditions of incubation should be isotonic to intracellular osmotic pressure, and this fact should receive due attention. Measurement of the volume of a solitary blastomer is fraught with difficulties of mechanical manipulations with it. With the development of laser microscopy methods: layer-by-layer scanning (microphotography) and 3D reconstruction of biological microobjects – it became possible to solve this problem.

Institute of Theoretical and Experimental Biophysics, the Russian Academy of Sciences, Pushchino, Russia. ***Address for correspondence:*** agpogorelov@rambler.ru. A. G. Pogorelov

MATERIALS AND METHODS

The study was carried out on F_1(CBA×C57Bl) mice. Two-cell embryos were collected as described previously [8]. Washed embryos were incubated in Dulbecco's medium. Embryos collected directly after isolation from the oviduct served as the control. The direction of osmotic shock was simulated by changing the concentration of NaCl in the incubation medium.

The fundamentals of the method based on cryofixation of biological tissue were described previously [7,8]. The initial step is snap freezing of the object in liquid propane (-188°C). Frozen specimens were lyophilized in vacuum (~10^{-5} Pa) at -100°C. After hypothermic dehydration was over, the dry object was embedded in medium based on epoxy resin (epon 812).

After resin thermopolymerization (60°C), the preparation was examined under an LSM 510 confocal microscope (Carl Zeiss). A series of optical sections in the vertical direction at a 2-μ step was obtained in transmitting light (scanning laser microscopy). The blastomer contour was sketched in the plane of each section and the 3D aspect of the embryonic cell was restored by the series of successive contours. The 3D

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TABLE 1. Changes in the Volume ($\times 10^3$ μ^3) of a Mouse Two-Cell Embryo Blastomer after Incubation under Different Osmotic Conditions (*M*±*m*)

NaCl concentration in Dulbecco's* medium	Duration of incubation in Dulbecco's medium, min				
	0	15	30	60	120
200 mM	55±4 (*n*=24)	38±3 (*n*=22)	37±4 (*n*=24)	37±2 (*n*=20)	37±4 (*n*=10)
140 mM	55±4 (*n*=24)	51±3 (*n*=24)	48±6 (*n*=22)	47±5 (*n*=22)	53±6 (*n*=12)
60 mM	55±4 (*n*=24)	102±9 (*n*=24)	88±12 (*n*=22)	57±7 (*n*=22)	52±4 (*n*=10)

Note. *n*; number of measured embryos per group. *Status directly after removal of the embryo from the oviduct is taken for its initial status.

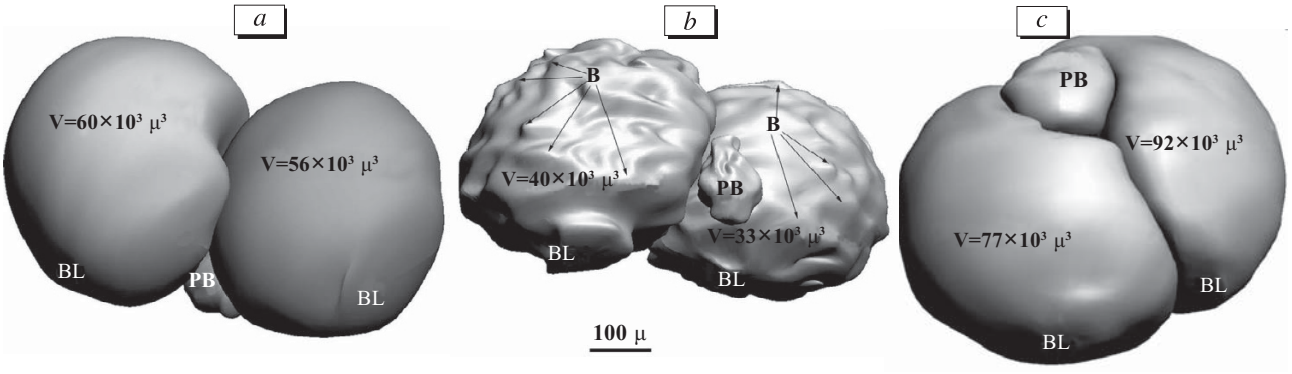

Fig. 1. Embryo after 3D reconstruction. *a*) directly after isolation of the embryo from oviduct; *b*) 120-min incubation in medium containing 200 mM NaCl; *c*) 15-min incubation in medium with 60 mM NaCl. BL: blastomer; PB: polar body; B: blebs; V: blastomer volume

reconstruction was carried out using a standard graphic editor, for example, 3ds max, allowing measurements of volumic characteristics of a reconstructed object.

RESULTS

The results of quantitative microtomography of a mouse two-cell embryo under different osmotic conditions are shown in Figure 1.

The volume of the blastomer was changing depending on the osmotic factor direction. Hyperosmotic shock involved also modification of the cell shape with emergence of characteristic protrusions, "blebs" (Fig. 1, *b*). The technology used in our study not only visualizes the 3D image of the early embryo, but allows evaluation of its separate compartment volumes. Comparative data on changes in the embryonic cell volume during osmotic stress are summed up in Table 1.

Analysis of the data obtained for the blastomer exposed to hyperosmotic medium (200 mM NaCl) showed that the embryonic cell behaved like an osmometer. After rapid 30% shrinkage, the cell volume reached the plateau and did not restore. Common Dulbecco's solution (140 mM NaCl) also causes a lasting ~18% reduction of cell volume. However, a trend to recovery of the initial cell status is observed during long culturing. These data indicate that the saline used in the study is slightly hypertonic and the changes in blastomer volume induced by it are reversible.

It seems that osmotic stress under conditions of "normal" saline is with time compensated for by the adaptive mechanisms; this effect was demonstrated for some differentiated cells. Activation of ion transport systems on the membrane explains also the abnormal osmotic behavior of the blastomer under hypotonic conditions (Table 1). In this case the phase of rapid swelling of the cytoplasm, caused by water entry into the cell, is followed by a regulatory phase of volume recovery. Hence, the microtomography technology, developed on the basis of laser scanning microscopy, can be used for quantitative evaluation of the reaction of mammalian early embryonic cell to osmotic shock.

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