Analysis of Changes in Cell Volume of a Mouse Early Embryo Exposed to Osmotic Shock

M. A. Pogorelova[1,2], D. V. Goldshtein[3,4], A. G. Pogorelov[2,5], and V. A. Golichenkov[1]

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 3, pp. 169-172, July, 2009
Original article submitted March 20, 2009

The impact of the osmotic component of the incubation medium for the volume of mouse early embryonic cell was studied by laser scanning microscopy. Common Dulbecco's medium caused a prolonged hyperosmotic effect. Adaptive phase of regulatory compensation for the osmotic shock was observed under hypotonic conditions. From these data, water permeability of the blastomer membrane is evaluated as 0.4 μ/(min×atm).

Key Words: *two-cell mouse embryo; osmotic stress; 3D-reconstruction; laser scanning microscopy; cell volume regulation*

Osmotic shock disorders embryo development [6,18]. Changes in the isotonic conditions can be caused by variations of osmotic characteristics of the solution and/or mediated through chemical inactivation of membrane proteins forming water channels and ion transport mechanisms [11]. For this reason, selection of incubation medium deserves special attention, because the parameters of this medium should be isotonic to the cells incubated in it.

In cell technologies, for example, in cryopreservation or embryonic cell enucleation, the volume characteristics are used as an integral parameter describing the trauma inflicted to the embryo during the experiment. Changes in cell volume are evaluated qualitatively by visual inspection or pseudoquantitatively [9]. In this latter case, the oocyte is assumed to be spherical and the cell volume is estimated by the effective diameter (section area), measured in the equatorial plane focus. Importantly, the choice of the focus plane is very subjective.

This method is incorrect after completion of the very first cell cycle, when the blastomer shape and section area become variables depending on the spatial orientation of the embryo during observation. For this reason, the development of approaches to direct measurements of the volume of an early embryo and its compartments [3] is an important problem. We used the technology of quantitative microtomography by laser scanning microscopy for evaluation of the osmotic effect on two-cell mouse embryo.

MATERIALS AND METHODS

The study was carried out on F_1(CBA×C57Bl) mice. The animals were kept under standard conditions (20±2°C, granulated PK-121-2 fodder, and free access to water). Water, fodder, and flooring were replaced daily without preliminary sterilization. Two-cell embryos were obtained as described previously [1]. Washed embryos were incubated in Dulbecco's medium. Two-cell embryos collected directly after isolation from the oviduct served as the control. Osmotic shock was created by modifying NaCl concentration in the incubation medium.

Methods of the preparation making based on snap cryofixation of the biological tissue were described previously [2,12-14,16]. The initial step is cryofixa-

[1]M. V. Lomonosov Moscow State University; [2]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino; [3]ReMeTex Firm; [4]Medical Genetic Center, Russian Academy of Medical Sciences, Moscow; [5]Pushchino State University, Russia. ***Address for correspondence:*** agpogorelov@rambler.ru. A. G. Pogorelov

0007-4888/09/1481-0152

tion of the object in liquid propane (-188°C). Frozen specimens were freeze-dried in vacuum (~10^{-5} Pa) at -100°C on an MBA-5 device (Balzers). After low-temperature dehydration, the dry specimen was embedded in Epon-812 [10]. Impregnated embryos were transferred into the wells of a plate and the medium was polymerized at 59°C.

The volume of a blastomer was measured by quantitative microtomography [4,15]. The preparation was examined under an LSM 510 confocal microscope (Zeiss). Vertically stacked optical sections with a 2-μ step were obtained in the transmitted light mode. Because of poor contrast of the resultant digital image, each section was additionally processed according to a universal algorithm. The blastomer contour was drawn on the microphotograph of the embryo in each optical section plane and the 3D image was reconstructed from a series of contours. 3D reconstruction was carried out using standard graphic editor.

RESULTS

The optical section plane of a two-cell mouse embryo was evaluated by laser scanning microscopy (Fig. 1).

3D reconstruction of the embryo was carried out after processing a series of successive optical sections (Fig. 2).

This technology of laser microtomography makes it possible not only to visualize the 3D object, but also to measure its volume characteristics quantitatively (Fig. 3).

The data (Fig. 3, curve 1) indicate abnormal osmotic behavior of the embryonic cell under hypotonic conditions. Rapid swelling of the cytoplasm, induced by water entry into the cell, is followed by a regulatory

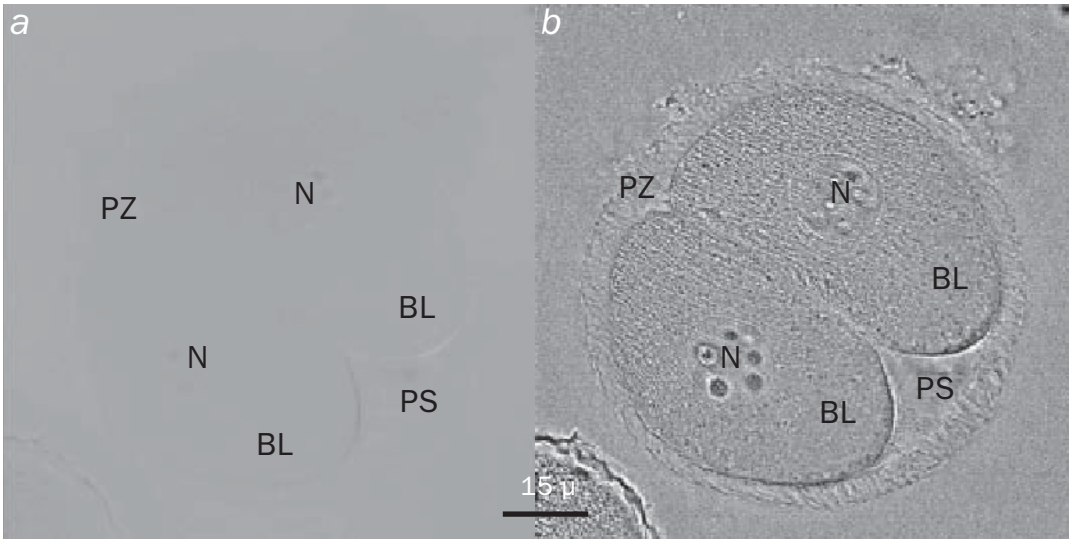

Fig. 1. Typical picture of a two-cell mouse embryo in the optical section plane from a vertical pile, obtained by laser scanning microscopy. a) unstained embryo; b) unstained embryo after mathematical processing in a graphic editor. BL: blastomer; N: nucleus; PS: perivitelline space; PZ: pelucide zone.

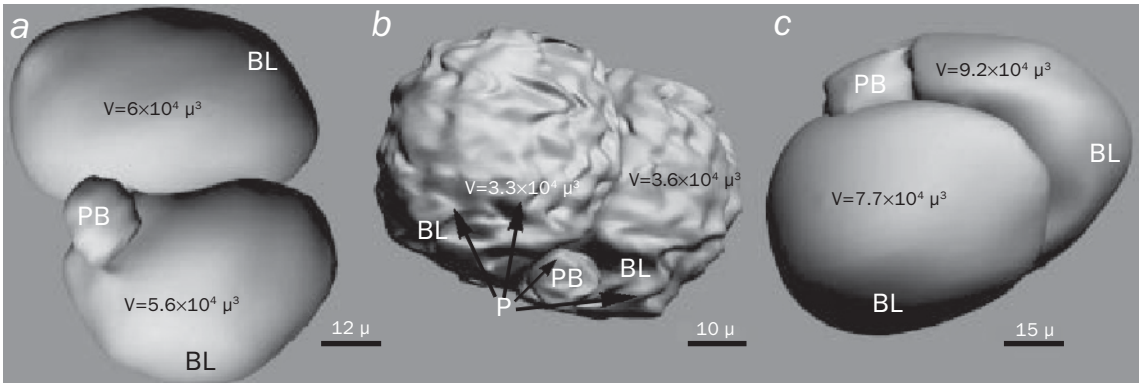

Fig. 2. Typical picture of a two-cell mouse embryo after three-dimensional reconstruction by a series of optical sections obtained by laser scanning microscopy. a) embryo after isolation from the oviduct; b) embryo after incubation in Dulbecco's hyperosmotic medium; c) embryo after short-term incubation in Dulbecco's hypotonic medium. BL: blastomer; PB: polar body; P: protrusions; V: blastomer volume.

phase of the volume restoration. Presumably, this effect is caused by the adaptive reaction to hypo-osmotic stress, demonstrated for differentiated cell cultures [17]. Using an equation for evaluation of membrane permeability for water, we get the value for the respective Lp coefficient:

$$Lp=(DVt/DP)/(t\times S),$$

where Lp is effective permeability for water, DV is the increment of cell volume during t moment, DP is the difference between the osmotic pressure under isotonic and hypo-osmotic conditions, and S is cell surface area.

The surface area of two-cell mouse embryo blastomer was evaluated previously (~10^4 μ^2) [15]. The Lp coefficient of 0.4 μ/(min×atm) can be calculated from the volume of embryonic cell during maximum swelling. The result is close to the value estimated for mouse mature oocytes in the maturing second cell division metaphase [5,8].

Common Dulbecco's solution (Fig. 3, curve 2) causes a smooth ~18% reduction of the volume, which indicates that "normal" saline is slightly hypertonic. Importantly that even a short-term osmotic shock causes disorders in the development of an embryo [6,7,18]. A trend to recovery of the initial status of the cell is observed during long culturing. Similarly as with a hypo-osmotic exposure, this behavior is explained by activation of the compensatory systems of ion transport on the membrane.

Hyperosmotic medium causes uncompensated changes in the blastomer volume (Fig. 3, curve 3). In this case, the embryonic cell behaves as a common osmometer. After rapid 30% shrinking, the cell volume reaches the plateau and is not restored. Hence, the laser microtomography technology is a direct method for quantitative evaluation of the volume of an individual cell of early mammalian embryo. The kinetics of the blastomer reaction to osmotic shock was demonstrated by this technology on a two-cell mouse embryo. We think that there are good grounds to use the laser quantitative microtomographic approach in studies of other types of isolated cell systems, for example, suspended or cultured cells.

The study was supported by the Russian Foundation for Basic Research (grants No. 08-04-01761a and Bel-08-04-90053a).

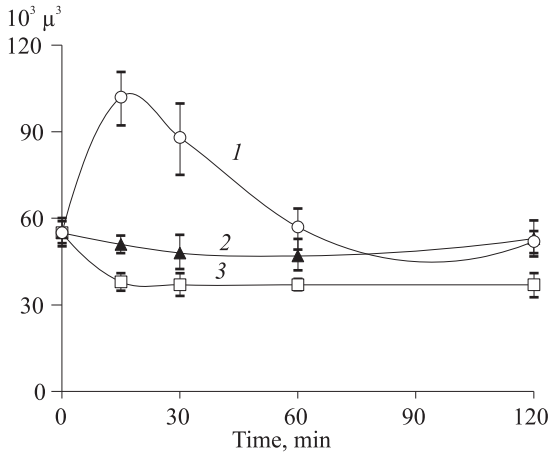

Fig. 3. Changes in the volume of a two-cell mouse embryo blastomer during osmotic stress of different direction. 1) Dulbecco's hypo-osmotic medium with 60 mM NaCl; 2) Dulbecco's common medium with 140 mM NaCl; 3) Dulbecco's hyperosmotic medium with 200 mM NaCl.

REFERENCES

1. M. Mank, *Developmental Biology of Mammals (Methods)* [in Russian], Moscow (1990).
2. A. G. Pogorelov and D. V. Goldshtein, *Kletochn. Tekhnol. Biol. Med.,* **2**, 84-91 (2006).
3. A. G. Pogorelov, G. M. Kantor, N. Yu. Sakharova, *et al.*, *Tsitologiya*, **47**, 686-691 (2005).
4. M. A. Pogorelova, V. A. Yashin, A. G. Pogorelov, and A. A. Golichenkov, *Dokl. Akad. Nauk*, **418**, 712-714 (2008).
5. Y. Agca, J. Liu, J. J. McGrath, *et al.*, *Cryobiology*, **36**, No. 4, 287-300 (1998).
6. J. D. Biggers, J. A. Lawitts, and C. P. Lechene, *Mol. Reprod. Dev.*, **34**, No. 4, 380-390 (1993).
7. B. Fong, P. H. Watson, and A. J. Watson, *BMC Dev. Biol.*, **7**, No. 2, 1-16 (2007).
8. D. Y. Gao, C. T. Benson, C. Liu, *et al.*, *Biophys. J.*, **71**, No. 1, 443-450 (1996).
9. D. Y. Gao, J. J. McGrath, J. Tao, *et al.*, *J. Reprod. Fertil*., **102**, No. 2, 385-392 (1994).
10. A. M. Glauert and P. R. Lewis, *Biological Specimen Preparation for Transmission Electron Microscopy*, New Jersey (1998).
11. I. I. Katkov and A. G. Pogorelov, *Cryo Letters*, **28**, No. 6, 409-427 (2007).
12. A. G. Pogorelov, B. L. Allachverdov, I. V. Burovina, *et al.*, *J. Microsc.*, **162**, 255-269 (1991).
13. A. G. Pogorelov, I. I. Katkov, and V. N. Pogorelova, *Cryo Letters*, **28**, No. 6, 403-408 (2007).
14. A. G. Pogorelov, I. I. Katkov, E. I. Smolyaninova, and D. V. Goldshtein, *Ibid.*, **27**, No. 2, 87-98 (2006).
15. A. G. Pogorelov and V. N. Pogorelova, *J. Microsc.*, **232**, No. 1, 36-43 (2008).
16. A. G. Pogorelov, V. N. Pogorelova, N. V. Repin, and I. A. Mizin, *Scanning Microscopy. Quantitative Electron Probe Microanalysis with a Wavelength Dispersive Spectrometer,* Suppl. 8, 101-108 (1994).
17. B. B. Shank, H. M. Rosenberg, and C. Horowitz, *J. Cell. Physiol.*, **82**, No. 2, 257-265 (1973).
18. L. J. Van Winkle, N. Haghighat, and A. L. Campione, *J. Exp. Zool*., **253**, No. 2, 215-219 (1990).