Quantitative LSM microtomography of early mouse embryo subjected to hypoosmotic shock

M. A. Pogorelova[1,2], V.N. Pogorelova[2], and V. A. Golichenkov[1]

1. Biological Faculty, Moscow State University, Leninskie gory 1/12, Moscow, 119991, Russia
2. Institute of Theoretical and Experimental Biophysics, RAS, Pushchino, Moscow province, 142290, Russia

pogm2007@rambler.ru
Keywords: early mouse embryo, LSM, 3-DR, hypotonic shock

Cell volume alteration triggers a multitude of intracellular regulatory mechanisms including membrane potential [1], ion concentrations [2], gene expression [1], cell death [3], protein synthesis [4]. The early embryo membrane is highly permeable for water, that makes the cell very sensitive to osmotic shock [5]. At what manner cellular volume of early mammalian embryo is appreciably altered by the extracellular solution is not understood. Originally, a little attention was paid to this direction since there were no effective ways to determine the volume of embryonic cells because of their small sizes.

Osmotic adaptation in a blastomere of two-cell mouse embryo has been studied employing the direct measurement of cellular volume with laser scanning microscopy (LSM) followed by three-dimensional reconstruction (3-DR). The keeping of the intact volume (shape) of the embryo compartments was based on freeze-drying technique [6, 7]. Several variants of hypotonicity were created by changes of NaCl contents in the incubation medium. After cryofixation in liquid propane and subsequent low-temperature dehydration, the embryo was immersed in the Epon medium. A Z-stack of optical slices at a step of 1 μm between the layers was obtained in a confocal microscope (Zeiss 510, Germany). 3-DR was performed in the 3ds max medium. Figure 1 illustrates the comparative results of the reconstruction of the embryo after hypoosmotic shock.

Embryonic cells subjected to hypotonic conditions were found to behave as non ideal osmometer over a wide range of tonicities. It was shown that blastomeres exposed to hypotonicity first swell and then gradually return to initial volume (Figure 2). During swelling phase cells were perfectly defined by a van't Hoff equation with the water permeability coefficient of 0.4 micron$\cdot$ min$^{-1}\cdot$ atm^{-1}, that is close to the value measured for metaphase II mouse oocytes [8, 9]. For shrinking phase an empirical equation has been fitted to the data.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-265, © Verlag der TU Graz 2009

1. F. Lang et al., Phys. Rev. **78 (**1998) pp. 247-278.
2. C.D. Cone Jr, J. Theor. Biol. **30** (1971) pp. 151-181.
3. G.M. Cohen et al., Biochem. J. **28** (1992) pp. 331-334.
4. K. Anbari et al., Mol. Reprod. Dev. **35** (1993) pp. 24-28.
5. A.D.C. Macknight, Ren. Physiol. Biochem. **11** (1988) pp. 114-141.
6. A.G. Pogorelov et al., J. Microsc. **232** (2008) pp. 36-43.
7. A.G. Pogorelov et al., CryoLet. **28** (2007) pp. 403-408.
8. Y. Agca et al., Cryobiol. **36** (1998) pp. 287-300.
9. D.Y. Gao et al., Biophys. J. **71** (1996) pp. 443-450.

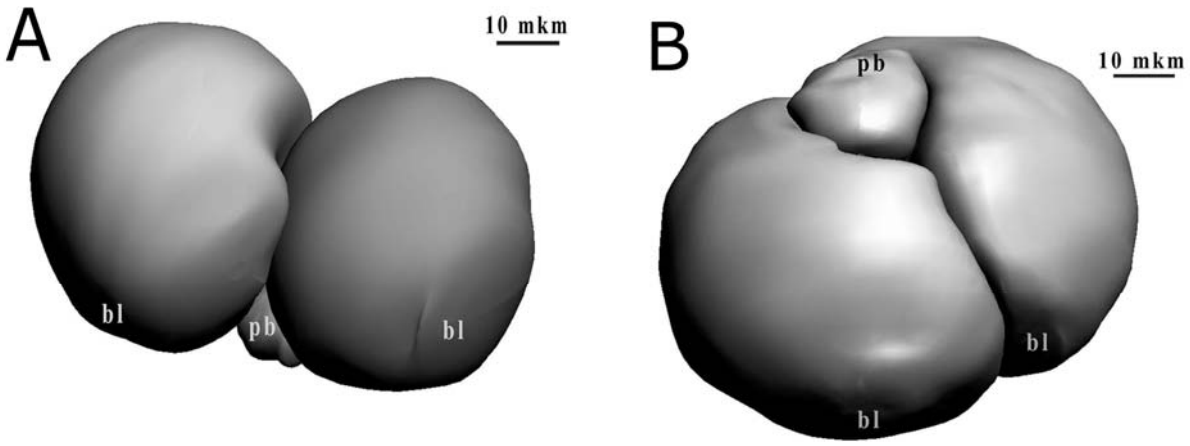

Figure 1. Two-cell mouse embryos images obtained with 3-D reconstruction. (A) Control embryo cryofixed immediately after extraction from oviduct; (B) 15 min incubation in hypotonic conditions; b- blebs, bl- blastomere, pb-polar body.

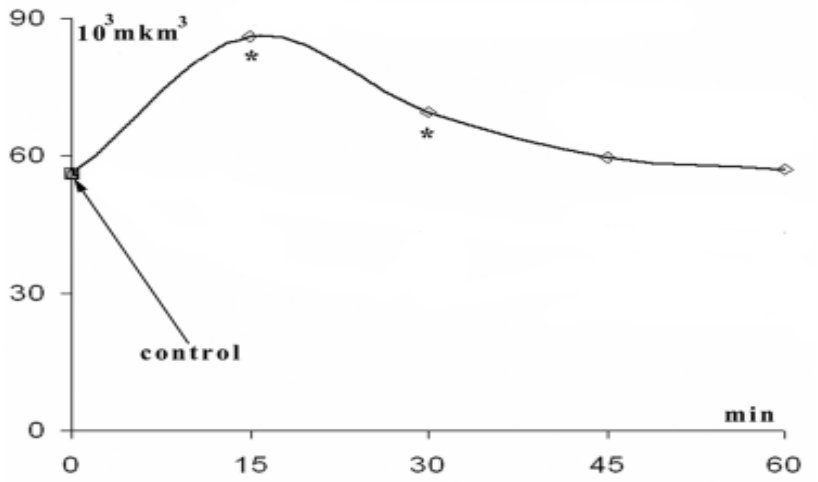

Figure 2. Changes of blastomere volume in 2-cell mouse embryos during exposure to Dulbecco`s solution with 70 mM NaCl. “Control” – embryos cryofixed immediately after flushing from oviduct. (*) $P < 0.05$ vs. control value (Student’s *t*-test).

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-265, © Verlag der TU Graz 2009