CryoLetters **41 (4)**, 209-215 (2020)

PHYSICAL-MATHEMATICAL MODEL OF SUBSTANCE REDISTRIBUTION BETWEEN THE CELL AND ITS HYPERTONIC SOLUTION ENVIRONMENT OF PENETRATING CRYOPROTECTANTS WITH RELEVANCE TO MEMBRANE POTENTIAL

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Abstract

BACKGROUND: The redistribution of basic ions between the cell cytoplasm and its surrounding medium due to osmotic action affects transmembrane potential and plasma membrane integrity at all stages of low temperature preservation. **OBJECTIVE:** To develop a physical-mathematical model describing the redistribution of osmotically active solutes between the cell and its hypertonic solutions of penetrating cryoprotectants that enables the calculation of kinetic changes in cell volume, cryoprotectant and ion concentrations, as well as the cell transmembrane potential during cell equilibration with cryoprotectant solutions. **MATERIALS AND METHODS:** The study has modeled the mass transfer process of mouse oocytes upon exposure to 1.5 M DMSO and 1,2-Propanediol (1,2-PD) solutions. **RESULTS:** Equations for changes of the normalized volume as well as intracellular concentrations of DMSO, 1,2-PD, potassium, sodium, chlorine and transmembrane potential have been obtained in a dimensionless form. The membrane permeability coefficients for DMSO and 1,2-propanediol have been determined and compared with the data of Paynter et al (5). **CONCLUSION:** The study shows that the incorporation of transmembrane ion movement and electrical potential change in the mathematical model leads to lower values of mouse oocyte membrane permeability coefficients for water and cryoprotectants in comparison with data determined by the traditional model.

Keywords: mouse oocyte, water transport, ion transport, transmembrane electrical potential

INTRODUCTION

Cell viability upon cryopreservation is affected by many factors at the stages from cell preparation, freezing by cooling and subsequent warming. During cryopreservation, the ion and water homeostasis in cells are disrupted. Such disturbances may occur at various stages during cryopreservation and are caused by dehydration and rehydration of cells, release from/entry into the cytoplasm of major ions which provide the cell ion balance. These processes may affect the cell transmembrane potential (2), which may lead to an imbalance of electrostatic lipid-lipid, lipid-protein and protein-protein interactions that play a critical role in maintaining the membrane structural integrity and function (7). One of the factors for cell injury upon cryopreservation is membrane damages due to the altered electric field from the ion redistribution between the extracellular and intracellular medium during cell exposure to cryoprotectant solutions and subsequent freezing (8, 9). The ion redistribution may be caused by the change in total volume of

osmotically active substances on each side of the membrane as well as the corresponding ions.

Methods for determining the coefficients of cell membrane permeability for cryoprotectants and water based on the Kedem-Kachalsky theory (3) (i.e., the cell volume excursion during their equilibration in hypertonic solutions) do not take into account the concentration change in ions inside and outside the cells.

The present work is to develop a physical-mathematical model for the redistribution of osmotically active substances between a cell and the hypertonic solutions of the penetrating cryoprotectants. This model allows to calculate the normalized cell volume, cryoprotectant concentrations and basic ion concentrations as well as cell transmembrane potential during a cell exposure to cryoprotectant solutions.

PHYSICAL/MATHEMATICAL MODEL

The equation to define the ion flow through the unit of membrane area is presented in (4):

$$j = Sp_m\left[C_m^{out}\exp\left(-\frac{z_m F\Delta\varphi}{2RT}\right) - C_m^{in}\exp\left(\frac{z_m F\Delta\varphi}{2RT}\right)\right], \quad [1]$$

where j is the flow of particles (mol/sec), S the membrane surface area (m^2), p_m cell membrane permeability coefficient for m-th type of ions (m/s), C_m^{out} and C_m^{in} the extra- and intracellular ion concentrations (mol/ m^3) and z_m the ion valence. F is the Faraday constant (96485.3365 C/mol), R the universal gas constant (8.3144598 J/mol K), T the temperature (K), and $\Delta\varphi$ the difference in electric potential between membrane outer and inner surfaces (V).

This Eqn can be presented as follows:

$$\frac{dN_m}{dt} = Sp_m\left[C_m^{out}\exp\left(-\frac{1}{2}z_m\Delta\varphi^*\right) - C_m^{in}\exp\left(\frac{1}{2}z_m\Delta\varphi^*\right)\right], \quad [2]$$

where N_m is the number of moles of m-th component in intracellular medium, t time s), $\Delta\varphi^* = (F/RT)\varphi$ is the dimensionless difference of membrane electrical potential. It is known that transmembrane electrical potential is determined by the Goldman-Hodgkin-Katz equation as described in (2):

$$\Delta\varphi = -\frac{RT}{F}\times \ln\frac{p_K C_K^{in} + p_{Na}C_{Na}^{in} + p_{Cl}C_{Cl}^{out}}{p_K C_K^{out} + p_{Na}C_{Na}^{out} + p_{Cl}C_{Cl}^{in}}, \quad [3]$$

where K, Na and Cl are the indices for potassium, sodium and chlorine, respectively. Assuming that the valence of each of main ions (chlorine, potassium and sodium) z_m=1, and introducing the notation, $f = exp(\Delta\varphi^*/2)$ the Eqn [2] takes the following form:

$$\frac{dN_m^{in}}{dt} = \frac{Sp_m}{f}\left(C_m^{out} - C_m^{in} f^2\right) \quad [4]$$

The concentration of the m-th ions in extra-cellular medium at the current time can be calculated by the formula:

$$C_m^{out} = \frac{A}{V^{out}(0) + (V_c(0) - V_n) - (V_c - V_n)}, \quad [5]$$

where $A = V^{out}(0)C_m{}^{out}(0) + [V_c(0) - V_n]C_m{}^{in}(0) - (V_c - V_n)C_m{}^{in}$, $V^{out}(0)$ is the volume of extracellular medium per cell at initial moment of time (m^3), $C^{out}(0)_m$ the extracellular concentration of the *m-th* ions at initial moment of time, (mol/m^3), $V_c(0)$ the cell volume at initial moment of time (m^3); V_c the cell volume at the current time, (m^3); V_n the volume of intracellular osmotically inactive substances (m^3), $C_m^{in}(0)$ the concentration of the m-th ions inside the cell at initial moment of time, (mol/m^3), C_m^{in} the concentration of chlorine ions inside a cell at the current time, (mol/m^3).

We introduce the following notations:

total volume of solution per cell,

$$V_F = V^{out}(0) + (V_c(0) - V_n)$$

$$H = V_c(0)/V_F,$$

$$\alpha = V_n/V_c(0),$$

$$V_c - V_n = \nu_w N_w + \sum \nu_m N_m + \nu_{cp} N_{cp}$$

where w, m and cp are the indices for water, the *m-th* ions and cryoprotectant, respectively; ν_w, ν_m i ν_{cp} the volume of one mole water, the *m-th* ions and cryoprotectant (mol/m^3), respectively; N_w, N_m i N_{cp} number of moles of water, the *m-th* ions and cryoprotectant inside a cell at the current time (mol), respectively, and

$$\overline{N}_w = \frac{\nu_w N_w}{V_F},\ \overline{N}_m = \frac{\nu_m N_m}{V_F},\ \overline{N}_{cp} = \frac{\nu_{cp} N_{cp}}{V_F}$$

are the dimensionless number of moles. So, Eqn [5] is as follows:

$$C_m^{out} = \frac{B}{1-(\bar{N}_w + \sum \bar{N}_m + \bar{N}_{cp})}, \qquad [6]$$

where B denotes

$$B = (1-H+\alpha H)C_m^{out}(0) + H(1-\alpha)C_m^{in}(0) - (\bar{N}_w + \sum \bar{N}_m + \bar{N}_{cp})C_m^{in}.$$

If we introduce the following notations:

$$E_m = (1-H+\alpha H)C_m^{out}(0) + H(1-\alpha)C_m^{in}(0);$$

$$G_m = \bar{N}_w + \sum \bar{N}_{m-1} + \bar{N}_{cp},$$

we will get

$$C_m^{out} = \frac{E_m - (G_m + \bar{N}_m)C_m^{in}}{1-(G_m + \bar{N}_m)}. \qquad [7]$$

Considering Eqn [7], Eqn [4] has the form:

$$\frac{dN_m^{in}}{dt} = \frac{Sp_m}{f} \times \left[\frac{E_m - (G_m + \bar{N}_m)C_m^{in}}{1-(G_m + \bar{N}_m)} - f^2 C_m^{in}\right]. \qquad [8]$$

After some transformations and taking

$$C_m^{in} = \frac{N_m^{in}}{V_C - V_n} = \frac{N_m^{in}/V_F}{G_m + \bar{N}_m} = \frac{\bar{N}_m/v_m}{G_m + \bar{N}_m},$$

we will get

$$\frac{dN_m^{in}}{dt} = \frac{Sp_m}{f} \times \frac{E_m + \left[(f^2-1)(G_m + \bar{N}_m) - f^2\right]\frac{\bar{N}_m}{v_m(G_m + \bar{N}_{ml})}}{1-(G_m + \bar{N}_m)}. \qquad [9]$$

By multiplying both sides of this equation by v_m/V_F and introducing $d\tau_m = \frac{Sp_m dt}{fV_F}$, we obtain the differential equation in dimensionless form:

$$\frac{d\bar{N}_m}{d\tau_m} = \frac{E_m v_m (G_m + \bar{N}_m) + K}{\left[1-(G_m + \bar{N}_m)\right](G_m + \bar{N}_m)} \qquad [10]$$

where K denotes $K = \left[(f^2-1)(G_m + \bar{N}_m) - f^2\right]\bar{N}_m$.

Opening the brackets and introducing the notation: $A_1 = f^2 - 1$; $B_1 = E_m v_m - f^2 + (f^2-1)G_m$; $C_1 = E_m v_m G_m$; $A_2 = -1$; $B_2 = 1 - 2G_m$; $C_2 = G_m - G_m^2$ and separating variables, we obtain an integral equation:

$$\int_{\bar{N}_m(0)}^{\bar{N}_m} \frac{A_2\bar{N}_m^2 + B_2\bar{N}_m + C_2}{A_1\bar{N}_m^2 + B_1\bar{N}_m + C_1} d\bar{N}_m = \int_0^{\tau_m} d\tau_m, \qquad [11]$$

where $\bar{N}_m(0)$ and $\bar{N}_m$ are the dimensionless number of moles of the m-th ions at the initial moment of time and the current time, respectively. Integrating the equation [11], we get the following dependence of dimensionless number of moles of the m-th ions inside the cell on the time of equilibration:

$$\frac{A_2(B_1^2 - 2A_1C_1) - A_1B_1B_2 + 2A_1^2C_1}{2A_1^2\sqrt{B_1^2 - 4A_1C_1}} \times \left[ln\left|\frac{2A_1\bar{N}_m + B_1 - \sqrt{B_1^2 - 4A_1C_1}}{2A_1N_m + B_1 + \sqrt{B_1^2 - 4A_1C_1}}\right| - ln\left|\frac{2A_1\bar{N}_m(0) + B_1 - \sqrt{B_1^2 - 4A_1C_1}}{2A_1\bar{N}_m(0) + B_1 + \sqrt{B_1^2 - 4A_1C_1}}\right|\right] + \frac{A_1B_2 - A_2B_1}{2A_1^2}\left[ln\left|A_1\bar{N}_m^2 + B_1\bar{N}_m + C_1\right| - ln\left|A_1\bar{N}_m^2(0) + B_1\bar{N}_m(0) + C_1\right|\right] + \frac{A_2}{A_1}\left[\bar{N}_m - \bar{N}_m(0)\right] = \tau_m. \qquad [12]$$

Eqn [12] is a system of equations for calculating the amount of main ions (Cl^-, Na^+ and K^+) inside the cell at the current time.

To determine the rate of water flow across a cell membrane during transmembrane transfer of solutes we use equation presented in (9):

$$\frac{dN_w}{dt} = -Sp_w \sum (C_k^{out} - C_k^{in}), \qquad [13]$$

where p_w is the cell membrane permeability coefficient for water molecules; C_k^{out} and C_k^{in} the concentration of osmotically active solutes outside and inside a cell (chlorine, sodium and potassium). The extracellular concentration of each substance is determined by the formula:

$$C_k^{out} = \frac{D}{V^{out}(0) + (V_c(0) - V_n) - (V_c - V_n)},$$

where D and intracellular concentration are

$$D = V^{out}(0)C_k^{out}(0) + (V_c(0) - V_n)C_k^{in}(0) - (V_c - V_n)C_k^{in}; \; C_k^{in} = \frac{N_k^{in}}{V_c - V_n};$$

N_k^{in} is the number of moles of osmotically active substance inside a cell. Performing the same transformations as described above, we get:

$$\frac{dN_w^{in}}{dt} = -Sp_w\left\{\frac{\left[V_F - (V^{in}(0) - V_n)\right]\sum C_k^{out}(0)}{V_F - (v_w N_w^{in} + \sum v_k N_k^{in})} + \right. \quad [14]$$
$$\left. + \frac{(V^{in}(0) - V_n)\sum C_k^{in}(0) - V_F \sum C_k^{in}}{V_F - (v_w N_w^{in} + \sum v_k N_k^{in})}\right\}.$$

Multiplying both sides of Eqn [14] by v_w/V_F and introducing the following notations

$$E_w = (1 - H + \alpha H)\sum C_k^{out}(0) +$$
$$+ H(1-\alpha)\sum C_k^{in}(0);$$
$$\tau_w = -\frac{Sp_w t}{V_F};$$
$$G_w = \sum \overline{N}_k,$$

we get the equation in dimensionless form:

$$\frac{d\overline{N}_w}{d\tau_w} = \frac{E_w v_w \overline{N}_w + E_w v_w G_w - v_w \sum \frac{\overline{N}_k}{v_k}}{-\overline{N}_w^2 + (1-2G_w)\overline{N}_w + G_w - G_w^2}. \quad [15]$$

Further, introducing the notations:

$$A_3 = E_w v_w$$
$$B_3 = E_w v_w G_w - v_w\left(\sum \frac{\overline{N}_k}{v_k}\right)$$
$$A_4 = -1$$
$$B_4 = 1 - 2G_w$$
$$C_4 = G_w - G_w^2$$

and separating the variables with subsequent integrating, we can get the following equation:

$$\int_{\overline{N}_w(0)}^{\overline{N}_w} \frac{A_4\overline{N}_w^2 + B_4\overline{N}_w + C_4}{A_3\overline{N}_w + B_3} d\overline{N}_w = \int_0^{\tau_w} d\tau_w \quad [16]$$

After integrating this equation we get:

$$\frac{A_4}{2A_3}\left[\overline{N}_w^2 - \overline{N}_w^2(0)\right] + \left(\frac{B_4}{A_3} - \frac{A_4 B_3}{A_{31}^2}\right) *$$
$$* \left[\overline{N}_w - \overline{N}_w(0)\right] + \left(\frac{A_4 B_3}{A_3^3} - \frac{B_3 B_4}{A_3^2} + \frac{C_4}{A_{31}}\right) + \quad [17]$$
$$+ \frac{C_4}{A_3}\left(\ln\left|A_3\overline{N}_w + B_3\right| - \ln\left|A_3\overline{N}_w(0) + B_3\right|\right) = \tau_W.$$

When equilibrating cells in a penetrating cryoprotectant solution, the movement rate of cryoprotectant through the cell membrane can be calculated by the equation presented in (1):

$$\frac{dN_{cp}^{in}}{dt} = Sp_{cp}\left(C_{cp}^{out} - C_{cp}^{in}\right). \quad [18]$$

The cryoprotectant concentrations in the medium and inside a cell at the current time are:

$$C_{cp}^{out} = \frac{V^{out}(0)C_{cp}^{out}(0)}{V^{out}(0) + (V_c(0) - V_n) - (V_c - V_n)} +$$
$$+ \frac{(V_c(0) - V_n)C_{cp}^{in}(0) - (V_c - V_n)C_{cp}^{in}}{V^{out}(0) + (V_c(0) - V_n) - (V_c - V_n)},$$

and $C_{cp}^{in} = \frac{N_{cp}^{in}}{V_c - V_n}$, respectively.

Substituting these values into the Eqn [18] and following the same transformations as described above, we obtain the differential equation in dimensionless form:

$$\frac{d\overline{N}_{cp}}{d\tau_{cp}} = \frac{(v_{cp}E_{cp} - 1)\overline{N}_{cp} + v_{cp}E_{cp}G_{cp}}{-\overline{N}_{cp}^2 + (1-2G_{cp})\overline{N}_{cp} + G_{cp} - G_{cp}^2}, \quad [19]$$

Table 1. Data used for calculations (6).

K^+ ions inside oocyte C_K^{in}, mol/m³	204
Na^+ ions inside oocyte C_{Na}^{in}, mol/m³	83
Cl^- ions inside oocyte C_{Cl}^{in}, mol/m³	68
K^+ ions outside oocyte C_K^{out}, mol/m³	6
Na^+ ions outside oocyte C_{Na}^{out}, mol/m³	141
Cl^- ions outside oocyte C_{Cl}^{out}, mol/m³	120
Oocyte membrane permeability coefficient to K^+ ions P_K, m/sec	8×10^{-10}
Oocyte membrane permeability coefficient to Na^+ ions P_{Na}, m/sec	1.2×10^{-9}
Oocyte membrane permeability coefficient to Cl^- ions P_{Cl}, m/sec	1.6×10^{-8}

Table 2. Oocyte membrane permeability coefficients for water (p_w) and cryoprotectants (p_s).

Protectant	p_w, $\times10^{-5}$ m/s	p_s, $\times10^{-7}$ m/s	Reference
DMSO	1.5227	3.7	(5)
	1.45	2.45	our data
1,2-PD	1.261	4.3	(5)
	0.875	3.02	our data

where $\tau_{cp} = \frac{Sp_{cp}t}{V_F}$;

$G_{cp} = \overline{N}_w + \sum \overline{N}_m$;

$$E_{cp} = (1 - H + \alpha H) C_{cp}^{out}(0) +$$
$$+ H(1-\alpha) C_{cp}^{in}(0).$$

If we introduce the following notations

$$A_5 = \nu_{cp} E_{cp} - 1$$

$$B_5 = \nu_{cp} E_{cp} G_{cp}$$

$$A_6 = -1$$

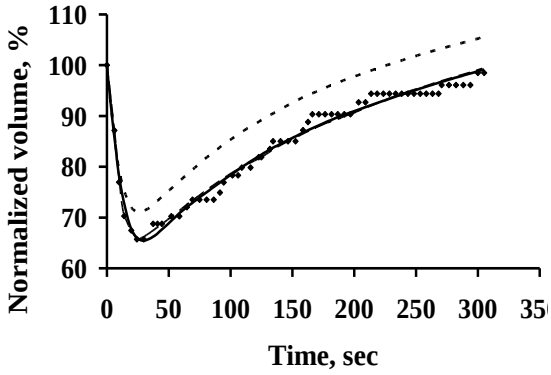

Figure 1. Dependence of mouse oocyte normalized volume on the equilibration time in 1.5 M DMSO solution. Symbols are experimental data and double dash line (— —) is the theoretical curve as reported by Paynter et al (5). Short dash line (---) is the theoretical curve according to the developed model using the values of oocyte membrane permeability coefficients as mentioned (5), where single dash line (—) is the theoretical curve obtained using the oocyte membrane permeability coefficients as determined by the developed model).

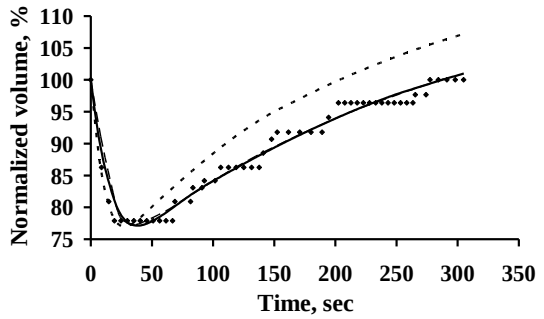

Figure 2. Dependence of mouse oocyte normalized volume on the equilibration time in 1.5 M 1,2-Propandiol solution. Symbols are experimental data and double dash line (— —) is the theoretical curve as reported by Paynter et al (5). Short dash line (---) is the theoretical curve according to the developed model using the values of oocyte membrane permeability coefficients as mentioned (5), where single dash line (—) is the theoretical curve obtained using the oocyte membrane permeability coefficients as determined by the developed model).

$$B_6 = 1 - 2G_{cp}$$

$$C_6 = G_{cp} - G_{cp}^2 \text{ and}$$

afterwards separate the variables and proceed to integrate, we will get the integral equation:

$$\int_{\overline{N}_{cp}(0)}^{\overline{N}_{cp}} \frac{A_6 \overline{N}_{cp}^2 + B_6 \overline{N}_{cp} + C_6}{A_5 \overline{N}_{cp} + B_5} d\overline{N}_{cp} = \int_0^{\tau_{cp}} d\tau_{cp} \qquad [20]$$

.

After integrating, Eqn [20] for determining the dependence of dimensionless amount of cryoprotectant inside a cell on time of cell exposure to cryoprotectant solution is obtained thus:

$$\frac{A_6}{2A_5}\left[\overline{N}_{cp}^2 - \overline{N}_{cp}^2(0)\right] + \left(\frac{B_6}{A_5} - \frac{A_6 B_5}{A_5^2}\right) *$$
$$* \left[\overline{N}_{cp} - \overline{N}_{cp}(0)\right] + \left(\frac{A_6 B_5^2}{A_5^3} - \frac{B_5 B_6}{A_5^2} + \frac{C_5}{A_{51}}\right) * \qquad [21]$$
$$* \left[\ln\left|A_5 \overline{N}_{cp} + B_5\right| - \ln\left|A_5 \overline{N}_{cp}(0) + B_5\right|\right] = \tau_{cp}.$$

To determine the extra- and intracellular components as well as membrane electrical potential the system of nonlinear Eqns [3], [12]-[14], [17] and [21] must be solved. This system is numerically solved using the iteration method.

RESULTS AND DISCUSSION

The developed model is used to calculate the normalized volume of mouse oocytes during the equilibration in solutions of two penetrating cryoprotectants, DMSO and 1,2-Propandiol (1,2-PD), and to determine membrane permeability coefficients to water and cryoprotectants. In order to estimate the adequacy of the physical-mathematical model, it is applied to the experimental data of Paynter et al (5) and compared to the obtained mouse oocyte membrane permeability coefficients for water and two cryoprotectants with those coefficients reported previously (5). We have considered that the oocyte is placed in a 1 mL solution of penetrating cryoprotectant; the concentration of each of the solution components was homogeneous throughout the volume of both extra- and intracellular media. Table 1 shows the parameters used for the calculations (6).

The dependency of mouse oocytes normalized volumes on the equilibration time in cryoprotectant solutions (at 30°C) has been digitized using the Zeiss LSN Image Examiner software and is used for comparison.

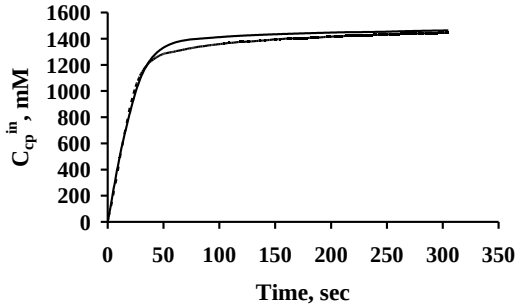

Figure 3. Dependence of cryoprotectant intracellular concentrations on the equilibration time in 1.5 M cryoprotectant solutions. Dash line is for DMSO and solid line is for 1,2-PD.

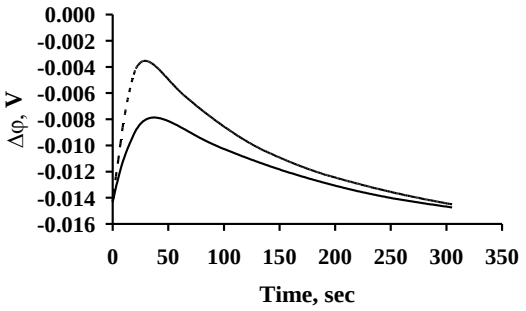

Figure 4. Dependence of transmembrane electrical potential of mouse oocyte on the equilibration time in 1.5 M solutions of cryoprotectants. Dash line is for DMSO and solid line is for 1,2-PD.

Experimental data as well as the calculations presented (5), have also been digitized. The results of the calculations are shown in Table 2 and Figs 1 – 5. As shown in Figs 1–2 the changes of mouse oocytes normalized volumes during equilibration in 1.5 M solutions of DMSO and 1,2-PD at 30°C according to our model using the determined permeability coefficients (5) resulted in overestimated values of normalized volume. When applying the permeability coefficients for water and cryoprotectant determined according to our model (Table 2), the dependence of normalized volume change quite well coincides with the theoretical curve obtained (5). If the calculations include the changes of intracellular and extracellular ion concentrations, as well as the transmembrane electrical potential, we obtain oocyte membrane permeability coefficients for water molecules in 1.5 M solution of DMSO lower by 4%, and in 1,5 M solution of 1,2-PD by 30%. The oocyte membrane permeability coefficient for DMSO is less by 30%, and by 35% for 1,2-PD.

Subsequently, all the calculations have been carried out when applying our own model for oocyte membrane permeability coefficients to water and cryoprotectants (Table 2) with the exposure time reported (5). Figure 3 shows the calculated dependencies of the 1,2-PD and DMSO intracellular concentrations on the equilibration time in 1.5 M solutions. According to the calculations, the concentration of 1,2-PD reached 1.463 M and DMSO reached 1.445 M, respectively. Figure 4 shows the time dependencies of the transmembrane electrical potential of the mouse oocyte during oocyte equilibration in 1,2-PD and DMSO solutions.

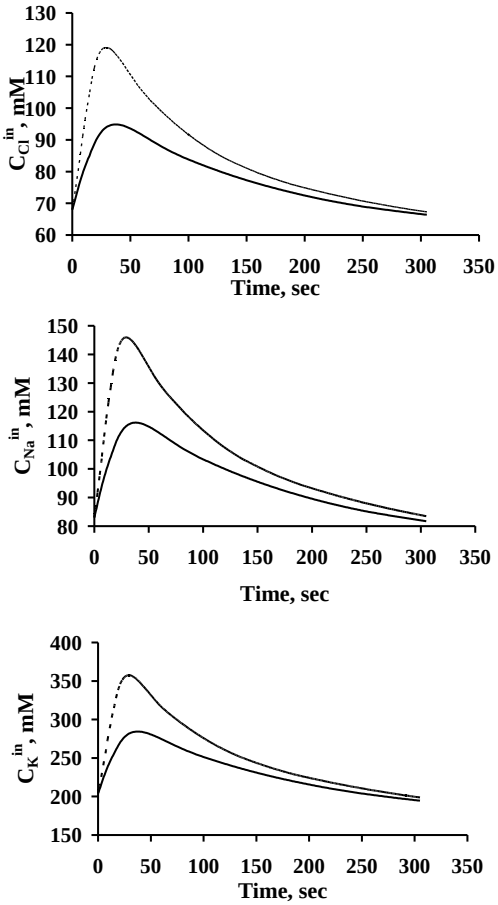

Figure 5. Dependence of oocyte ion intracellular concentrations on the equilibration time in 1.5 M cryoprotectant solutions (--- DMSO; — 1,2-PD). Upper, chlorine; middle, sodium concentration; lower, potassium concentration.

It can be seen that during mouse oocyte exposure to cryoprotectants solutions the membrane potential decreases by an absolute value and reaches a minimum of -3.5367 mV in 29 s in DMSO solution and -7.8708 mV in 38 s in 1,2-PD solution. The transmembrane potential

absolute value reaches its minimum in the same time as the cell volume does. Further, the electrical potential of the membrane increases and almost reaches its original value. In Figure 5 the dependencies of intracellular concentrations of chlorine, sodium and potassium ions on the time of mouse oocyte exposure to 1.5 M 1,2-PD and DMSO solutions are presented. The dependencies of intracellular ion concentrations on time is similar to the one of transmembrane electric potential presented in Figure 4.

Thus, we have suggested the physical-mathematical model of transmembrane transfer of substances considering the cell transmembrane electric potential, which, in turn, depends on the changes of basic ion concentrations inside and outside a cell. This model is in good agreement with experimental data on the change in the relative volume of mouse oocytes during exposure to hypertonic solutions of penetrating cryoprotectants. The model also provides more accurate permeability coefficients of mouse oocyte plasma membranes to molecules of water and cryoprotectants.

Acknowledgements: The authors would like to thank Professor Barry J Fuller for kindly permitting use of experimental data obtained in SJ Paynter et al (5) to perform the theoretical calculations and for discussing the work throughout.

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