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Apoptotic Shrinkage of Lymphoid Cells: A Model of Changes in Ion Flux Balance[1]

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There are two apparent discrepancies in the current views on the mechanism of apoptotic cell shrinkage. A decrease in the sodium pump activity is supposed to be responsible for apoptotic decrease in cell K^+ content and degradation of the pump is confirmed by immunoblot analysis of the α- and β-subunits of sodium pump in cell membrane and by other methods [1–4]. A decrease of both Rb^+ and $^{22}Na^+$ fluxes via sodium pump was observed during apoptosis induced by staurosporine in lymphoid U937 cells in our own experiments. However, deficiency in the pumping rate of Na^+ and K^+ should lead to cell swelling, whereas shrinkage of cell is a hallmark of apoptosis [5, 6].

The second discrepancy is that the opening of the K^+ channels is believed to play a significant role in the loss of intracellular K^+ during apoptosis [7]. But the opening of these channels should lead to hyperpolarization of apoptotic cells [8], instead of depolarization, which is observed in experiments [2, 4].

In the present study, an integrated mathematical model of cell volume and ion balance regulation is considered, in order to find out what changes of the monovalent ion pathways across the plasma membrane could lead to changes in the ion and water balance in the apoptotic cells. These changes, e.g., in lymphoid cells, include a decrease in the pumping of Na^+ and K^+ across plasma membrane, a decrease in the intracellular K^+/Na^+ ratio, cell shrinkage, and cell depolarization. Apoptosis of the U937 cells caused by staurosporine was used as a prototype in our computer modeling of ion and water balance in a cell, but the data obtained are valid for a wide class of cells. Finally, we came to conclusion that the changes in more that one ion pathways through cell membrane should be responsible for changes in ion and water balance during apoptosis.

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Equations of ion and osmotic balance. A mathematical model of ion and water balance with respect to ion fluxes across the cell membrane was developed for erythrocytes in [9], and for other cells in [8, 10, 11]. Apoptosis as a whole is a non steady-state phenomenon. However, the ion and water balance in cells is achieved much faster than the apoptosis proceeds. Therefore, the terms with the time derivatives in ion and water flux equations can be neglected and the algebraic Eqs. (1)–(5) with the variable parameters can be used for modeling of the separate steps of apoptosis as in [8, 10, 11].

$$[Na^+]_i + [K^+]_i - [Cl^-]_i + zA/V = 0, \quad (1)$$

$$[Na^+]_i + [K^+]_i + [Cl^-]_i + A/V = [Na^+]_0 + [K^+]_0 + [Cl^-]_0, \quad (2)$$

$$p_1(t)u\{([Na^+]_i e^u - [Na^+]_0)/(1 - e^u)\} - b\beta(t)[Na^+]_i + S_{NC} = 0, \quad (3)$$

$$p_2(t)u\{([K^+]_i e^u - [K^+]_0)/(1 - e^u)\} + (b/\gamma)\beta(t)[Na^+]_i = 0, \quad (4)$$

$$p_3(t)u\{([Cl^-]_i - [Cl^-]_0 e^u)/(1 - e^u)\} + S_{NC} = 0, \quad (5)$$

where the symport term S_{NC} is defined by the formula:

$$S_{NC} = q_{NC}(t)\{1 - [Na^+]_i[Cl^-]_i/[Na^+]_0[Cl^-]_0\}. \quad (6)$$

Equations (1)–(2) represent the electroneutrality of solutions separated by the cell membrane and the water-osmotic equilibrium between cell and medium. $[Na^+]_0$, $[K^+]_0$, $[Cl^-]_0$ and $[Na^+]_i$, $[K^+]_i$, $[Cl^-]_i$ are external and intracellular ion concentrations in cell water, mM; A is the intracellular content of impermeant anions, moles per cell; z is the average charge of these anions; V is the cell volume per cell (taken to be equal to the water content); u is the dimensionless transmembrane electrical potential difference, $u = FU/RT$, where U is the potential difference in mV. Equations (3)–(5) represent the balance of

influx and efflux for every species of ions through overall pathways. The first two terms in Eqs. (3)–(5) are fluxes through channels defined as in Goldman theory. The terms $b\beta(t)[Na^+]_i$ and $(b/\gamma)\beta(t)[Na^+]_i$ are Na^+ and K^+ fluxes via sodium pump, which depend on internal $[Na^+]_i$; and γ is the stoichiometric coefficient for K^+ and Na^+ transport by the pump. The coefficients b and β represent the intrinsic properties of the cell and the pump, respectively. It should be pointed out that using a nonlinear relationship between pump fluxes and concentrations of K^+ and Na^+ inside and outside of the cell does not change our conclusions in general. S_{NC} is the net flux of Na^+ and Cl^- through the complex of Na^+/H^+ and Cl^-/HCO_3^- - antiporters (termed hereinafter as a "symport"), S_{NC} is defined by (6), where $q_{NC}(t)$ is Na^+ influx representing the intrinsic properties of antiporters when the ion composition of the medium is constant. The 1 : 1 stoichiometry of the Na^+ and Cl^- symport is followed from the 1 : 1 stoichiometry of the transport of H^+ and HCO_3^- out of the cell which is obligatory when intracellular pH remains constant.

The solution of Eqs. (1)–(5) gives the values of five basic variables: $[Na^+]_i$, $[K^+]_i$, $[Cl^-]_i$, U, and V_C (cell volume V_C is a volume per mole of impermeant anions in the cell, $V_C = V/A$). The values of ion fluxes are obtained from basic variables. For practical use, it was convenient to introduce dimensionless coefficients of the integral channel permeability $p_{Na} = p_1/b$, $p_K = p_2/b$, $p_{Cl} = p_3/b$, and dimensionless Na^+ and K^+ fluxes via the pump *ENaP* (Na^+ efflux), *IKP* (K^+ influx), via symporter $Q_{NC}(t)$ (Na^+ influx), *ENaNC* (Na^+ efflux), and through channels *IKG* (K^+ influx). For this purpose, all terms of Eqs. (3)–(5) were divided by a "reference" Na^+ flux at $[Na^+]_i = 1$ mM, i.e. $\{b \times [1\ \text{mM}]\}$. The dimension and dimensionless values can be converted each into other by the formulas:

$$p_{Na} = p_1/b, \quad p_K = p_2/b, \quad p_{Cl} = p_3/b,$$

$$ENaP = -\beta(t)[Na^+]_i/[1\ \text{mM}],$$

$$ENaG = p_{Na}u\{[Na^+]_i/[1\ \text{mM}]\}e^u/(1 - e^u),$$

$$IKP = \{(1/\gamma)\beta(t)[Na^+]_i\}/[1\ \text{mM}],$$

$$IKG = -p_K u\{[K^+]_0/[1\ \text{mM}]\}/(1 - e^u),$$

$$Q_{NC}(t) = q_{NC}(t)/b[1\ \text{mM}]\},$$

$$ENaNC = \{-Q_{NC}(t)[Na^+]_i[Cl^-]_i/([Na^+]_0[Cl^-]_0)\}.$$

To correlate the experimental data with the data in Figs. 1, 2 the calculated values should be multiplied by $b \times [1\ \text{mM}]$. For the cells U937 $b = 0.33$ ml/g of cell protein/min, as Na^+ efflux via at $[Na^+]_i = 30$ mM equals 10 μmol/g protein/min.

Ion and water balance as a function of properties of channels and transporters carrying Na^+, K^+, and Cl^- across the cell membrane. Original data used as a prototype in modeling of cell ion and water balance were obtained in our experiments with the human lymphoma cell line U937 [6]. Apoptosis of these cells treated with staurosporine (1 *M*, 4h) was accompanied by 25–30% loss of cell water (volume), by a decrease in the K^+/Na^+ ratio from 4–5 to 1.5–2.5, while the total Na^+ flux and partial fluxes of Na^+ and K^+ through the pump (*IKP*, *ENaP*) decreased 2–4 times. It has been assumed that the apoptosis is associated with cell depolarization [2, 4].

Figure 1 shows changes in ion and water balance as a function of the pump activity for four cases: (1) symport is absent (dotted line, filled circles), (2) non-zero symport is constant (solid line without symbols), (3) degradation of the pump is accompanied by a decrease of symport (filled triangles), (4) degradation of the pump is accompanied by the opening of Cl^- channels (open triangles). It can be observed, that the K^+/Na^+ ratio is always decreasing. However, changes in the cell water content (cell volume) appear to be different in the compared cases. Pump degradation alone leads to an increase in cell water content, i.e., to cell swelling. Symport increases cell volume significantly. When symport is decreased, cell dehydration occurs, and this effect may override the swelling caused by the pump degradation.

Opening of the Cl^- channels decreases cell water content and can also lead to cell shrinkage in spite of the simultaneous degradation of the pump. Delay in a decrease of symport or in opening of Cl^- channels shifts the minimum of the cell volume, V_C, to the lower values of the pump activity β. Both the decrease of symport and the opening of Cl^- channels, combined with the pump degradation, can cause simultaneously a decrease of K^+/Na^+ ratio, a decrease of pump fluxes and cell shrinkage. The cases (3) and (4) appear to differ only when additional phenomena are taken into account, e.g. the changes of membrane potential. When cell shrinkage is caused by opening of Cl^- channels, the changes of membrane potential are more pronounced, than in the case when it is caused by a decrease of symport. So, measurement of the membrane potential can help to answer the question which of these two parameters, Q_{NC} or p_{Cl}, is responsible for the apoptotic cell shrinkage.

What are the changes in fluxes in the compared cases? The pump Na^+ efflux and K^+ influx (*ENaP* and *IKP*) are decreased in the studied range about 1.5–2 times, practically irrespective to the cause of shrinkage. In contrast, Na^+ influx is decreased more significantly when shrinkage is due to decreasing of symport, than in the case of the opening of Cl^- channels. This difference can indicate which parameter is changed, Q_{NC} or p_{Cl}.

Figure 2a shows changes in the ion and water balance as a function of the integral permeability of Cl^-, K^+ and Na^+ channels under the constant vs. decreasing pump activity and constant or zero symport. Opening of Cl^- channels leads to cell dehydration both at the constant and decreasing pump activity. However, no

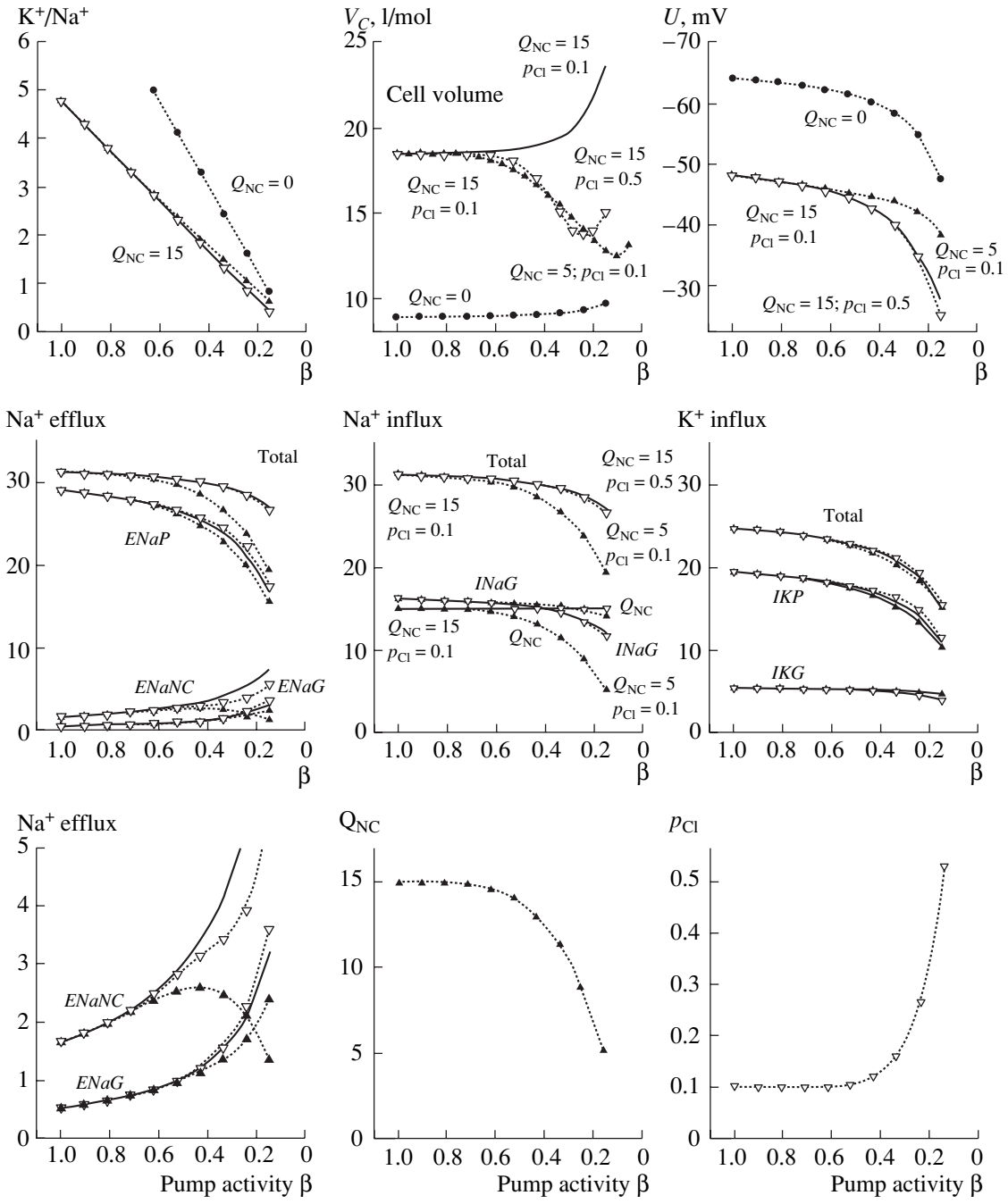

Fig. 1. Changes in ion and water balance due to a decrease of the Na^+, K^+-ATPase pump alone or with a simultaneous decrease of Na–Cl symport or opening of Cl^- channels. It is assumed that the pump activity parameter b is decreased linearly while Na–Cl symport parameter Q_{NC} and permeability of Cl^- channels p_{Cl} are changed nonlinearly, as shown on the two right layers. The data were obtained by numerical solution of Eqs. (1)–(5). At $[Na^+]_0$ = 150 mM, $[K^+]_0$ = 5 mM, $[Cl^-]_0$ = 155 mM, z = –1.5, J = 1.5, T = 310 K and fixed values of the permeability of Na^+ and K^+ channels, p_{Na} = 0.05; p_K = 0.5. Values of variable parameters are shown in indimentionless units.

changes in the Na^+/K^+ ratio, the membrane potential and Na^+ and K^+ fluxes occur when the opening of Cl^- channels takes place without decreasing of the pump. This illustrates that Cl^- channels presumably function as a cell water regulator [10–12].

Opening of K^+ channels under non-zero symport can lead to an apoptotic cell dehydration and a decrease in the K^+/Na^+ ratio at the same time, if the pumping decreases (Fig. 2b). However, under the constant symport, this should be accompanied by a hyperpolariza-

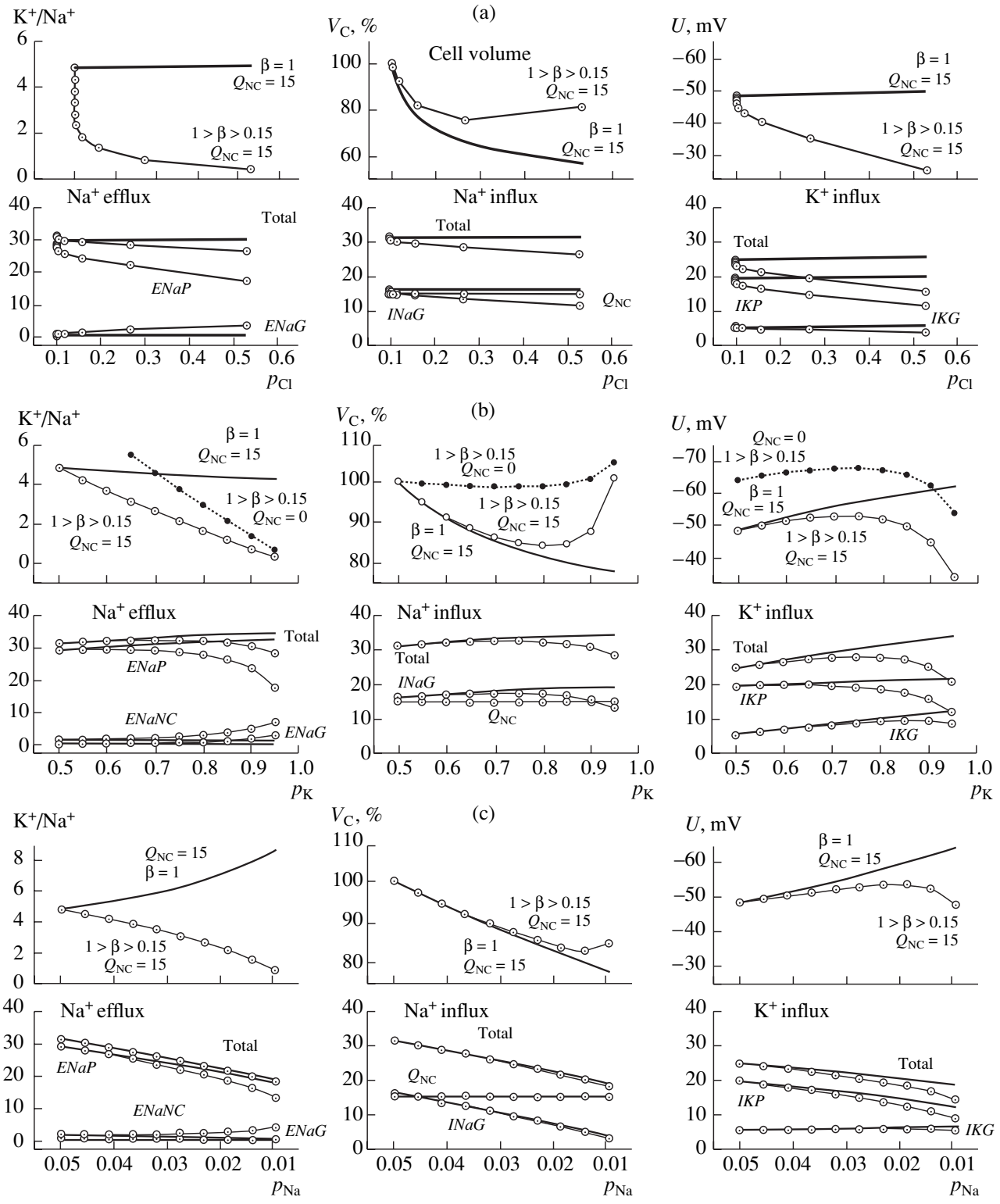

Fig. 2. Changes in ion and water balance as a function of integral permeability effect of Cl^-, K^+, and Na^+ channels under constant or decreasing pump activity in a system with constant or zero symport: (a) opening of Cl^- channels, (b) opening of K^+ channels, and (c) closing of Na^+ channels. The abscissa axis shows channel permeability coefficients p_{Cl}, p_K, and p_{Na}. Designations on the ordinate axis are the same as in Fig. 1. Solid line without symbols shows the case when the pump properties are not changed. Open circles show a decrease in the pump activity occurring simultaneously with changes in permeability of channels. Dotted line with filled circles shows the case when symport is absent. The range of changes in the parameters is shown on panels. Values of the variable parameters are indicated on abscissa; non-variable parameters were, respectively, $p_{Na} = 0.05$; $p_K = 0.5$; $p_{Cl} = 0.1$, $Q_{NC} = 15$. For other details see legend to Fig. 1. The data were obtained by numerical solution of Eqs. (1)–(5).

tion, in contrast to what is observed in the experiments [2, 4]. Cell dehydration can be caused by closing of Na^+ channels (Fig. 2c). However, the K^+/Na^+ ratio is increased by closing of Na^+ channels, if the pump activity is constant. Closing of Na^+ channels, combined with a decrease in the pump activity, leads to cell dehydration and a decrease in the K^+/Na^+ ratio at the same time, but in all cases closing of Na^+ channels should be associated with a hyperpolarization rather than with a depolarization of the cell. Closing of Na^+ channels in all cases should lead to a decrease of Na^+ influx and efflux. This is a peculiar feature of the changes of the cell ion and water balance caused by closing of the Na^+ channels.

In conclusion, the apoptotic cell dehydration, associated with a decrease of in the K^+/Na^+ ratio, cell depolarization and a decrease of in the K^+ and Na^+ pump fluxes, is theoretically possible, if the degradation of the Na^+, K^+-ATPase pump is accompanied by a decrease of the Na^+–Cl^- symport, or by opening of Cl^- channels. Which of the last two factors plays the dominant role in the apoptotic cell shrinkage can be determined by the analysis of the changes in the cell membrane potential and Na^+ fluxes. However, the experimental data along this line are rather fragmentary, because no attempt has been made yet to consider an integrated model of the ion and water balance in apoptotic cells. It is shown by electrophysiological methods, that the state of Cl^- channels during apoptosis can change, and that alteration of these channels by some drugs can affect the apoptosis [12, 13]. On the other hand, it was reported that Na^+/H^+ exchange during apoptosis was reduced [14, 15].

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REFERENCES

1. Nobel, C.S. and Aronson, J.K., van den Dobbelsteen, D.J., et al., *Apoptosis*, 2000, vol. 5, no. 2, pp. 153–163.
2. Düssmann, H., Rehm, M., Kogel, D., et al., *J. Cell Sci.*, 2003, vol. 116, no. 3, pp. 525–536.
3. Arrebola, F., Cañizares, J., Cubero, M.A., et al., *Apoptosis*, 2005, vol. 10, no. 6, pp. 1317–1331.
4. Franco, R., Bortner, C.D., and Cidlowski, J.A., *J. Membr. Biol.*, 2006, vol. 209, no. 1, pp. 43–58.
5. Bortner, C.D. and Cidlowski, J.A., *Europ. J. Physiol.*, 2004, vol. 448, no. 3, pp. 313–318.
6. Yurinskaya, V., Goryachaya, T., Guzhova, I., et al., *Cell Physiol. Biochem.*, 2005, vol. 16, nos. 4/6, pp. 155–162.
7. Burg, E.D., Remillard, C.V., and Yuan, J.X.-X., *J. Membr. Biol.*, 2006, vol. 209, no. 1, pp. 3–20.
8. Vereninov, A.A., Yurinskaya, V.E., and Rubashkin, A.A., *Dokl. Biol. Sci.*, 2004, vol. 398, no. 4, pp. 417–421 [*Dokl. Akad. Nauk*, 2004, vol. 398, no. 4, pp. 555–559].
9. Lew, V.L. and Bookchin, R.M., *J. Membr. Biol.*, 1986, vol. 92, no. 1, pp. 57–74.
10. Vereninov, A.A., Glushankova, L.N., and Rubashkin, A.A., *Tsitologiya*, 1995, vol. 37, no. 12, pp. 1151–1166.
11. Vereninov, A.A., Glushankova, L.N., and Rubashkin, A.A., *Tsitologiya*, 1997, vol. 39, no. 8, pp. 727–739.
12. Szabó, I., Gulbins, E., Apfel, H., et al., *J. Biol. Chem.*, 1996, vol. 271, no. 34, pp. 20465–20469.
13. Okada, Y., Shimizu, T., Maeno, E., et al., *J. Membr. Biol.*, 2006, vol. 209, no. 1, pp. 21–29.
14. Lang, F., Madlung, J., Bock, J., et al., *Eur. J. Physiol.*, 2000, vol. 440, no. 6, pp. 902–907.
15. Rich, I.N., Worthington-White, D., Garden, O.A., and Musk, P., *Blood*, 2000, vol. 95, no. 4, pp. 1427–1434.