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Research article

**SOLUTE-DEPENDENT ACTIVATION OF CELL MOTILITY
IN STRONGLY HYPERTONIC SOLUTIONS IN *Dictyostelium*
discoideum, HUMAN MELANOMA HTB-140 CELLS
AND WALKER 256 CARCINOSARCOMA CELLS**WŁODZIMIERZ KOROHODA^{1*}, MAGDALENA KUCIA^{1,2}, EWA
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Abstract: Published data concerning the effects of hypertonicity on cell motility have often been controversial. The interpretation of results often rests on the premise that cell responses result from cell dehydration, i.e. osmotic effects. The results of induced hypertonicity on cell movement of *Dictyostelium discoideum* amoebae and human melanoma HTB-140 cells reported here show that: i) hypertonic solutions of identical osmolarity will either inhibit or stimulate cell movement depending on specific solutes (Na⁺ or K⁺, sorbitol or saccharose); ii) inhibition of cell motility by hypertonic solutions containing Na⁺ ions or carbohydrates can be reversed by the addition of calcium ions; iii) various cell types react differently to the same solutions, and iv) cells can adapt to hypertonic solutions. Various hypertonic solutions are now broadly used in medicine and to study modulation of gene expression. The observations reported suggest the need to examine whether the other responses of cells to hypertonicity can also be based on the solute-dependent cell responses besides cell dehydration due to the osmotic effects.

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Abbreviations used: BSS – balanced salt solution; CME – coefficient of movement efficiency; EGTA – ethylene glycol bis(β-aminoethyl ether)-N,N-tetraacetic acid; HTB-140 – human melanoma cell line; SEM – standard error of the mean; TRITC – tetramethyl rhodamine iso-thiocyanate

Key words: Cell motility activation, Hypertonicity, Solute-dependent, Membrane interaction

INTRODUCTION

Some types of cell are exposed to a broad range of osmotic pressure fluctuations in their natural environment and are resistant to medium hypertonicity [1-4]. Strongly hypertonic solutions have often been used recently in the medical treatment of patient injuries [5-7] and to modify gene expression and enzyme activity [8, 9]. However, the knowledge of mechanisms by which the hypertonic solutions act on cells remains insufficient. The effects of hypertonicity on cells have been studied in numerous laboratories but the results obtained and their interpretations are often controversial, in particular, when the effects of hypertonicity on the motile activity of cells are analyzed. Some observations have suggested that tissue cells are unable to move or to produce pseudopodia when embedded in hypertonic solutions [10,11]. There are equally numerous observations of cells capable of adapting to hypertonicity, surviving, moving, and growing in strongly hypertonic solutions [12-15]. The problem of the influence of hypertonicity on cell motile activity is important since cell motility plays a pivotal role in embryogenesis, wound healing, and metastasis [16-20], and osmotic forces have been suggested to be involved in mechanisms of generation of forces behind cell movement [10,11]. The experiments reported here were performed to find the reasons for the discrepancy in opinions on the effects of hypertonicity on movement of eukaryotic cells. They were carried out on myxamoebae of *Dictyostelium discoideum*, which have often been used as model cells in research into mechanisms of cell movement, and on human HTB-140 melanoma tumor cells and Walker 256 carcinosarcoma cells, since cell motility has often been inferred during the process of metastasis.

MATERIAL AND METHODS

Cells

The amoebae of *Dictyostelium discoideum* strain AX-2 (ATCC24397) were cultured as described previously [21, 22]. The Hs294T human melanoma cell line (No: HTB-140) was obtained from American Type Culture Collection (ATCC, Rockville, USA). Melanoma cells were used for experiments between passages 5 and 15. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma), supplemented with 100 IU/ml penicillin, 10 µg/ml streptomycin and 50 µg/ml neomycin (Polfa, Tarachomin, Poland) in the presence of 10% heat-inactivated fetal calf serum (GIBCO). The cells were plated at an initial cell density of 2.5×10^4 cells per 25 cm² Corning flask; the medium was changed every 48 h and cells were cultured in a humidified atmosphere with 5% CO₂ at 37°C. Walker 256 carcinosarcoma cells were cultured as described [23].

Fluorescent staining

Actin staining was done according to the procedure described by Bereiter-Hahn and Kajstura [24]. In brief, cells were fixed and permeabilized in 3.7% paraformaldehyde/0.1% Triton X-100, and stained with TRITC-Phalloidin (Sigma Chemical Co., St. Louis, Mo.).

For microtubule staining cells were fixed in methanol and blocked with 3% bovine albumin in TBA (20 mM Tris, 150 mM NaCl, 2 mM ethylene glycol bis(β -aminoethyl ether)-N,N-tetraacetic acid (EGTA), 2 mM MgCl₂, and incubated with monoclonal anti-human microtubule antibody (Sigma) at a dilution of 1:500 for 45 min at 37°C. After being washed 3 times with TBA, cells were incubated with anti-mouse IgG antibody conjugated to TRITC (Sigma) for 45 min at 37°C, washed, and the fluorescent images were examined with a Leica DM-IRE 2 microscope [25].

Time-lapse monitoring of movement of individual cells

The images of migrating *D. discoideum* amoebae and HTB-140 cells were recorded and analyzed with computer-aided methods [21, 26]. *D. discoideum* amoebae were observed after spreading in a Petri dish and recorded for 20 minutes with an inverted Hund Wilovert S microscope, recorded with a Hitachi CCD camera, digitized and processed with programs written by P. Jochym and R. Tokarski as described previously [27]. Images were acquired every 2 s for 20 minutes and tracks of individual cells were generated. The cell trajectories were presented in circular diagrams with the starting point of each trajectory situated in the center of the diagram [26, 28, 29].

Scatter correlation diagrams of total trajectory and displacement lengths of *D. discoideum* amoebae were prepared as described [22, 26]. Random walk analysis was done as described by Gail [30] and [21, 31]. Trajectories were composed from 60 sequential centroid positions, and the program Mathematica was used for further processing. In order to determine the ability of amoebae to migrate after incubation in hypertonic medium, each recorded cell was contoured from the moment it stopped migration (t_0) and again after 2 or 8 hours of incubation. Cells were considered migrating if their projections after 30 minutes did not share any area with their projections at t_0 . The following parameters characterizing cell locomotion were computed for each cell or cell population:

- total length of the cell trajectory (μm), i.e. the sum of a sequence of n straight-line segments, each corresponding to the cell centroid translocation in a given time interval;
- the average speed of cell movement, calculated from the cell path in a given time;
- the total length of cell displacement (μm), i.e. the distance from the starting point to the final cell position;
- the coefficient of movement efficiency (CME), corresponding to the ratio of cell displacement to cell trajectory length. The CME would equal 1 for cells

moving persistently along one straight line in one direction and 0 for a random movement.

The analysis of HTB-140 human melanoma cell movement in DMEM was performed in the same way. The recording of moving cells started 2 h after cell seeding, when the cells were already spread on plastic. The cell trajectories were constructed from 60 subsequent cell centroid positions recorded for 60 min. with time intervals of 60 s with a CCD camera attached to an inverted Olympus IMT-2 microscope using a 20X objective, at 37°C. Fifty cell tracks were recorded under each of the experimental conditions tested. The cell trajectories were presented in circular diagrams with the starting point of each trajectory situated in the center of the diagram.

Locomotion of cells in hypertonic media

In research concerning the effects of hypertonic solutions on a variety of cell activities, carbohydrates are usually used as osmolytes [1, 11, 14]. Experiments with *D. discoideum* amoebae migrating in hypertonic solutions were carried out in either 2% KCl (w/w) (587.5 mOsm) or 2% NaCl (w/w) (670.5 mOsm), 5% sorbitol (w/w) (298.75 mOsm) or 9.5% saccharose (w/w) (334.75 mOsm), in Chalkley's salt solutions. Osmolarity of Chalkley's solution is 35.3 mOsm. Osmolarity of solutions was measured with a freezing point osmometer (Marcel os3000) which measures water activity. The Van't Hoff equation sometimes used in medical and biological works can be applied only to very diluted solutions and gases, and not to relatively concentrated solutions, as explained in textbooks of physical chemistry (for example, Kruyt and Overbeek in 1960 [32], Barrow in 1961 [33] or Katchalsky and Curran in 1965 [34]).

To study the effect of hypertonic conditions on movement of human melanoma HTB-140 cells, time-lapse recording started just after the control DMEM medium was replaced by the medium supplemented with saccharose or sorbitol and after 60, 120, and 180 minutes of incubation. The hypertonic solutions were prepared by addition of saccharose or sorbitol to the DMEM to increase the osmolarity from 308 to 650 and 620 mOsm, respectively. The reversibility of the effects of hypertonic conditions was observed 60 minutes after the hypertonic medium in which the cells were pre-incubated for 180 minutes was removed and the cells returned to the control DMEM medium. Cell viability was tested with trypan blue exclusion and FDA/ethidium bromide tests [35] and in all tested solutions exceeded 95%.

Statistical analysis

Statistical significance was determined using the non-parametric Mann-Whitney U-test with $p < 0.01$ considered to indicate significant differences.

RESULTS

D. discoideum amoebae were exposed to hypertonic solutions of 300 to 600 mOsm and cell responses were observed. The cells rapidly decreased their volume to equalize the osmotic pressure of the intracellular and external water. This osmotic cell shrinkage persisted for hours, but the cell's motile responses changed in a few minutes. Initially the amoebae ceased to move, but this was resumed depending on the osmolyte (K^+ or Na^+) used in the hypertonic solutions. *D. discoideum* amoebae in strongly hypertonic 2% KCl solution (587.5 mOsm) in distilled water, after a few minutes of shock response, resumed active movement for several hours. However, these cells in 2% hypertonic NaCl solution (670.5 mOsm) immediately stopped movement and remained motionless for more than 8 h.

D. discoideum amoebae reacted differently compared to amoebae of the proteus-chaos group, or animal tissue cells, to hypertonic salt solutions in which potassium instead of sodium dominated. In pure 2% potassium chloride solutions the larger *Amoeba proteus* cells became spherical with liquefied cytoplasm, and all organelles sedimented vertically. This response was fully reversible on return to Chalkley's salt solution, when the cells resumed motility, formed pseudopodia, and sol-gel transitions and streaming within their cytoplasm resumed. Thus, despite similar initial osmotic shrinkage of both cell types in the hypertonic solutions, the subsequent motile responses of the two types of amoebae were different. The cells subjected to 2% KCl solution not only continued locomotion but their movement was significantly accelerated when compared to their behavior in control Chalkley's or BSS salt growth solutions (88.6 mOsm).

It is known that sodium and potassium differently modify mechanical properties of cell membranes and molecular protein interactions, and these monovalent ion effects can be modulated by divalent cations [36]. Therefore in the next experiments we examined the effect of addition of $CaCl_2$ (to 3 mM final concentration) to 2% KCl and 2% NaCl solutions on motile activity of *D. discoideum* amoebae. The results of this experiment are shown in Fig. 1 and Tabs. 1 and 2. A further increase in external solution osmolarity by the addition of calcium chloride caused restoration of motile activity and locomotion in cells which earlier remained motionless for hours in 2% NaCl solution (Tab. 2). This was not accompanied by the restoration of the osmotically reduced cell volume but rather with cell attachment to the substratum and induction of pseudopodia.

The effects of hypertonic solutions containing sorbitol or saccharose on *D. discoideum* amoebae movement were examined using solutions of 5% sorbitol (298.75 mOsm) and 9.5% saccharose (334.75 mOsm) dissolved in Chalkley's salt solution. Hypertonic solutions of sorbitol accelerated cell movement more than saccharose solution and the results are shown in Fig. 2 and Tab. 3. The addition of $CaCl_2$ to a final concentration of 3 mM in the 19% saccharose solution (744.5 mOsm) in which amoebae were motionless restored

movement of amoebae, though their rate of locomotion was lower than in Chalkley's or BSS solutions (results not shown). All the above described effects of *D. discoideum* amoebae responses to hypertonic solutions of different composition were found to be fully reversible after return of the cells to normal growth media, i.e. Chalkley's or BSS salt solutions (data not shown).

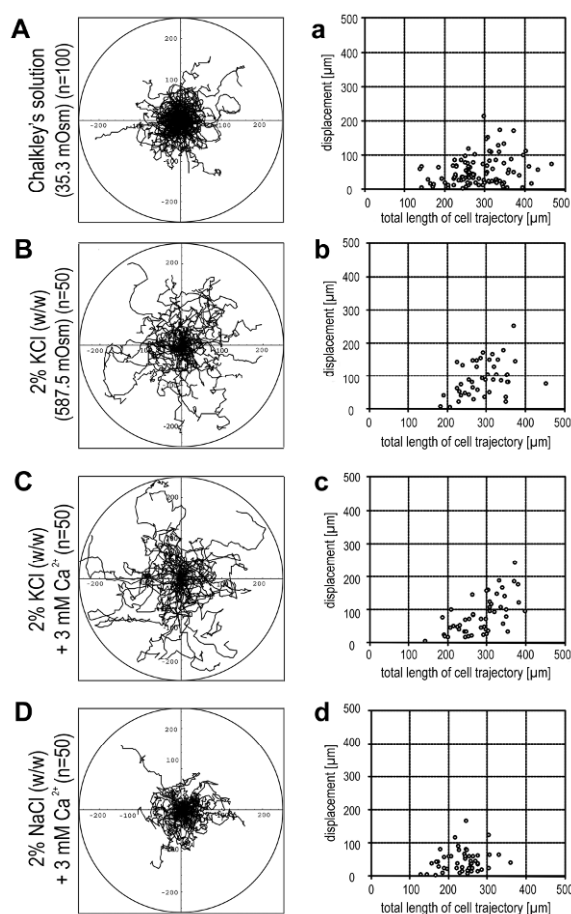


Fig. 1. The effect of addition of CaCl₂ to 2% KCl and 2% NaCl solutions on motile activity of *D. discoideum* amoebae. (A-D) The trajectories of *D. discoideum* cells migrating in control and hypertonic solutions. (a-d) Scatter diagrams show the correlation of the total length of cell trajectories and the total length of cell displacement. In circular diagrams the initial point of each trajectory was placed at the origin of the plot. Each trajectory was constructed from 60 successive positions of the cell centroid recorded at 20 s intervals. Cells before measurements were incubated for 8 h in control or experimental solutions.

D. discoideum amoebae in contrast to cells of vertebrate animals are often grown in media where potassium instead of sodium ions dominate [22, 37, 38,]. In their natural conditions, in soil, they are often exposed to fast changes in soil humidity

and salt solution osmolarity. Therefore the results of experiments carried out on *D. discoideum* amoebae demanded examination of whether they also concern tissue cells, in particular, because it has been shown that a high rate of cell motility in cancer cells correlates with their capacity to form metastases [18-20, 39]. The effects of hypertonic sorbitol and saccharose solutions in DMEM on movement of the human melanoma cell line HTB-140 were therefore analyzed.

Tab. 1. Percentage of *D. discoideum* amoebae migrating in hypertonic solutions of 2% KCl (w/w) or 2% NaCl (w/w) in Chalkley's solution supplemented with CaCl₂.

Time of incubation [h]	BSS	Chalkley's solution	2% KCl	2% KCl with CaCl ₂ (3 mM)	2% NaCl	2% NaCl with CaCl ₂ (3 mM)
2	98	92	83	82	0	13
8	100	100	100	93	0	90

Tab. 2. Values of parameters characterizing cell movement of *D. discoideum* amoebae in 2% NaCl (w/w) or 2% KCl (w/w) containing hypertonic solutions after 2 or 8 hours of incubation.

Parameters characterizing cell movement (\pm SEM)	Chalkley's solution	2% KCl		2% KCl with CaCl ₂ (3 mM)		2% NaCl	2% NaCl with CaCl ₂ (3 mM)
		Time of incubation [h]					
		2	8	2	8	8	8
Total length of cell trajectory [μ m]	283.4 \pm 6.6	257.0 \pm 8.1	290.3 \pm 8.3	171.8 \pm 5.6	285.1 \pm 8.37	0	253.5 \pm 6.7
Average speed of cell movement [μ m/min] ¹⁾	14.1 \pm 0.3	12.8 \pm 0.6	14.5 \pm 0.4	8.5 \pm 0.2	14.2 \pm 0.41	0	11.7 \pm 0.3
Total length of cell displacement [μ m]	54.4 \pm 4.1	35.5 \pm 3.9	92.8 \pm 7.8	29.3 \pm 3.8	83.07 \pm 7.83	0	45.8 \pm 4.6
Average rate of cell displacement [μ m/min] ²⁾	2.7 \pm 0.2	1.7 \pm 0.2	4.6 \pm 0.4	1.4 \pm 0.1	4.15 \pm 0.4	0	2.2 \pm 0.2
Coefficient of movement efficiency [CME] ³⁾	0.1 \pm 0.01	0.14 \pm 0.01	0.32 \pm 0.02	0.1 \pm 0.01	0.27 \pm 0.02	0	0.19 \pm 0.02

Note: Values are given as the mean \pm SEM. ¹⁾ The average speed of cell movement is defined as total length of cell trajectory/time of recording (20 min). ²⁾ The average rate of cell displacement is defined as length of final cell displacement/time of recording (20 min). ³⁾ Coefficient of movement efficiency – the ratio of cell displacement to cell trajectory length.

Quinones and Garcia-Castro [40] observed that HTB melanoma cell lines are characterized by very low migratory and invasive capacities in comparison to other human melanoma cell lines tested. Fig. 3 shows the trajectories of HTB-140 cells moving in control medium and in hypertonic medium supplemented with 9.5% saccharose (650 mOsm) recorded immediately after its addition (i.e. within the first 60 min) and 2 hours later, as presented in Tab. 4. In saccharose the hypertonic conditions caused a 3-4 fold increase in the rate of cell

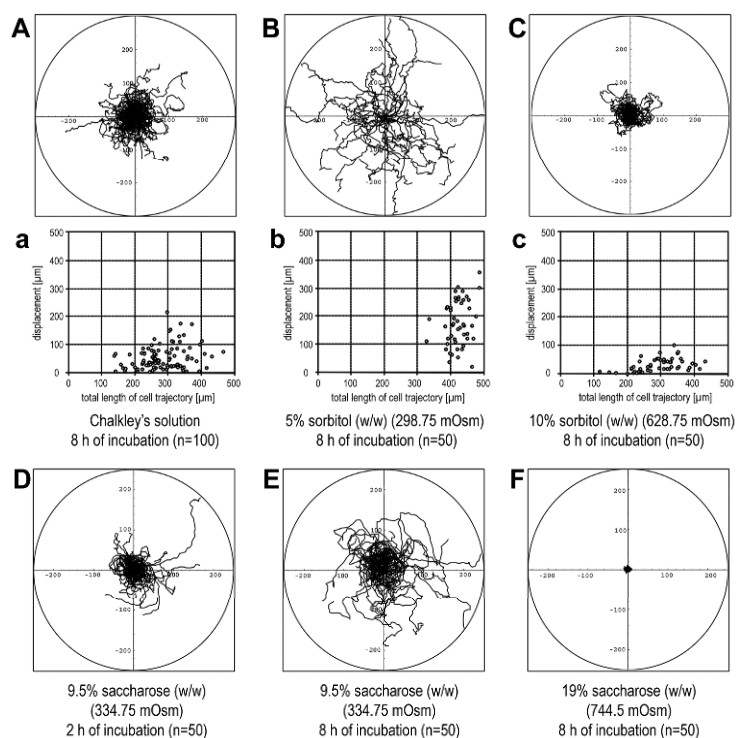


Fig. 2. The effects of hypertonic solutions containing sorbitol or saccharose on *D. discoideum* amoebae movement. (A-F) The trajectories of *D. discoideum* cells migrating under hypertonic conditions in Chalkley's solution with solute added. (a-c) Scatter diagrams showing correlation of the total length of cell trajectories and the total length of cell displacement. In circular diagrams the initial point of each trajectory was placed at the origin of the plot. Each trajectory was constructed from 60 successive positions of the cell centroid recorded at 20 s intervals.

movement and up to 5 fold increase in the length of cell displacement. In contrast to fast reversible changes of motility of *D. discoideum* cells after return to diluted medium, the acceleration of cell movement in HTB-140 cells persisted for the next 3 hours after the cells returned to isotonic control growth medium after earlier pre-incubation for 3 hours in the hypertonic medium containing saccharose. When 5% sorbitol in DMEM (630 mOsm) was used, a much weaker acceleration of HTB-140 cells was observed. On return to the control medium the cells after 3 h again moved slowly, showing more transient effects of the hypertonic sorbitol-enriched medium than the saccharose-enriched medium on cell motility. This was correlated with the transient effects of hypertonic sorbitol solutions on cell growth. HTB-140 cells did not proliferate in media enriched either with saccharose or sorbitol for 48 hours but after such pre-incubation and return to the control medium they grew at a similar rate as in the control growth medium without pretreatment with the hypertonic solutions (Fig. 4).

Tab. 3. Values of parameters characterizing cell movement of *D. discoideum* amoebae in sorbitol or saccharose containing Chalkley's solution after indicated time of incubation.

Parameters characterizing cell movement (\pm SEM)	Chalkley's solution (35.3mOsm)	5% sorbitol in Chalkley's solution (298.75 mOsm)			9.5% saccharose in Chalkley's solution (334.75 mOsm)		
		Time of incubation [h]					
		2	8	24	2	8	24
Total length of cell trajectory [μ m]	283.4 \pm 6.6	374.6 \pm 12.5	424.5 \pm 5.1	371.7 \pm 8.2	230.9 \pm 8.5	337.3 \pm 9.2	251.7 \pm 9.9
Average speed of cell movement [μ m/min]	14.1 \pm 0.3	18.7 \pm 0.6	21.2 \pm 0.2	18.5 \pm 0.4	11.5 \pm 0.4	16.8 \pm 0.4	12.5 \pm 0.5
Total length of cell displacement [μ m]	54.4 \pm 4.1	37.4 \pm 4.3	181.2 \pm 12.6	164.6 \pm 10.6	41.4 \pm 6.2	79.4 \pm 8.7	103.8 \pm 9.0
Average rate of cell displacement [μ m/min]	2.7 \pm 0.2	1.8 \pm 0.2	9.0 \pm 0.6	8.2 \pm 0.5	2.0 \pm 0.3	4.5 \pm 0.4	5.1 \pm 0.4
Coefficient of movement efficiency [CME] ³⁾	0.19 \pm 0.01	0.1 \pm 0.01	0.42 \pm 0.03	0.2 \pm 0.01	0.17 \pm 0.02	0.24 \pm 0.02	0.38 \pm 0.03

Note: Values are given as the mean \pm SEM. ¹⁾ The average speed of cell movement is defined as total length of cell trajectory/time of recording (20 min). ²⁾ The average rate of cell displacement is defined as length of final cell displacement/time of recording (20 min). ³⁾ Coefficient of movement efficiency – the ratio of cell displacement to cell trajectory length.

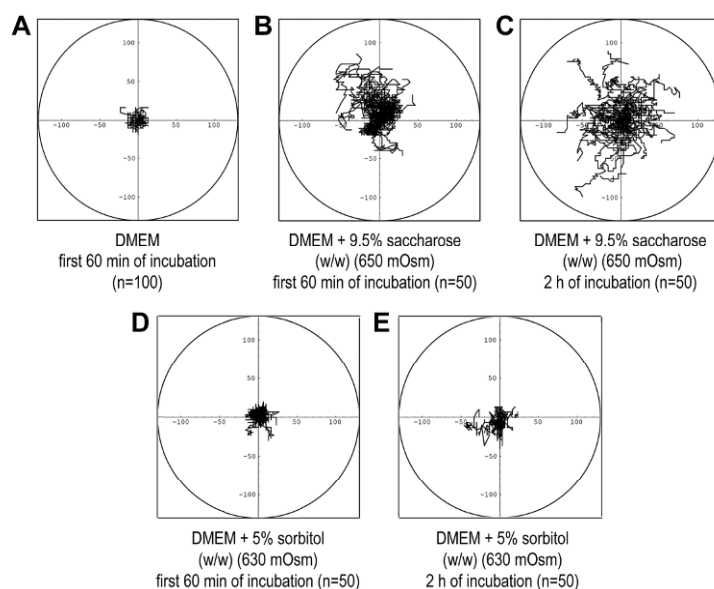


Fig. 3. The trajectories of human melanoma HTB-140 cells locomoting under control and hypertonic conditions displayed in circular diagrams with the initial point of each trajectory placed at the origin of the plot. The images were recorded for 60 min and the positions of the cell centroid were determined at 60-s time intervals.

Tab. 4. Values of parameters characterizing cell movement of human melanoma HTB-140 cells in control DMEM medium enriched with 9.5% saccharose (w/w) (650 mOsm) or 5% sorbitol (w/w) (630 mOsm).

Parameters	Time of incubation in hypertonic medium [h]						
	Control medium (CM)	0–1 + saccharose	2 + saccharose	3 after return to CM	0–1 + sorbitol	2 + sorbitol	3 after return to CM
Total length of cell trajectory [μm]	63.3 \pm 4.5	257.8 \pm 7.6 *	210.4 \pm 11.7*	239.1 \pm 10.6*	137.5 \pm 15.8*	234.8 \pm 8.5*	86.4 \pm 7.7*
Average speed of cell movement [$\mu\text{m}/\text{min}$] ¹⁾	1.0 \pm 0.1	4.3 \pm 0.1*	3.5 \pm 0.2*	3.9 \pm 0.2*	2.2 \pm 0.2*	4.2 \pm 0.1*	1.4 \pm 0.1*
Total length of cell displacement [μm]	7.1 \pm 0.5	30.7 \pm 2.5*	47.1 \pm 3.5*	31.9 \pm 3.1*	8.8 \pm 0.9*	13.9 \pm 0.9	9.9 \pm 0.9*
Average velocity of cell displacement [$\mu\text{m}/\text{min}$] ²⁾	0.1 \pm 0.01	0.5 \pm 0.04*	0.7 \pm 0.06*	0.5 \pm 0.05*	0.15 \pm 0.01*	0.23 \pm 0.02*	0.1 \pm 0.02*
Coefficient of movement efficiency (CME) ³⁾	0.16 \pm 0.02	0.13 \pm 0.01*	0.27 \pm 0.02*	0.16 \pm 0.02*	0.13 \pm 0.02*	0.06 \pm 0.01*	0.15 \pm 0.02*
Speed (S) [$\mu\text{m}/\text{h}$] ⁴⁾	1.67	7.01	4.03	11.61	4.85	4.16	3.29
Persistence in direction with time (P)	0.07	0.66	0.42	0.03	0.01	0.04	0.03
Diffusion constant (R) [$\mu\text{m}^2/\text{h}$] ⁴⁾	0.39	65.02	13.7	8.09	0.47	1.39	0.65

Note: Values are given as the mean \pm SEM, * statistically significant at $p < 0.05$. ¹⁾ The average speed of cell locomotion is defined as total length of cell trajectory/time of recording (60 min) ²⁾ The average velocity of cell displacement is defined as length of final cell displacement/time of recording. ³⁾ The ratio of cell displacement to cell trajectory length. CME would equal 1 for a cell moving persistently along one straight line in one direction and 0 for a random movement (Friedl *et al.*, 1993; Wójcicki *et al.*, 1995). ⁴⁾ These parameters were obtained by fitting a theoretical model of random motion to data consisting of the mean square displacements of the cells according to the method of Dunn. The fitting function is given by $\langle d^2 \rangle = 2S^2(PT - P^2(1 - \exp(-T/P)))$ and $R^2 = 2S^2P$, where T is time (Paddock and Dunn 1986).

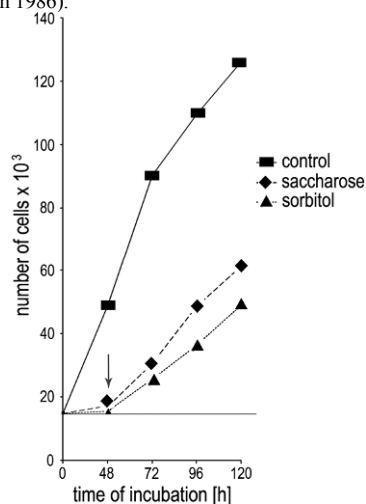


Fig. 4. Kinetics of growth of HTB-140 human melanoma cells in control medium (solid line) and after 48 h pre-incubations in hypertonic solutions of saccharose in DMEM (dash line) (650 mOsm) or sorbitol in DMEM (dotted line) (630 mOsm). The arrow indicates the change of the medium.

The above presented results show that hypertonic solutions of saccharose strongly accelerate movement of HTB-140 cells. The cells' cytoskeleton and organization of F-actin, as observed after phalloidin staining of actin and antibody staining of microtubule cytoskeleton in hypertonic saccharose solutions, assumed architecture similar to that described in keratinocytes [41-43] (Fig. 5 and 6). In fast locomoting HTB-140 cells in hypertonic saccharose solution we observed very intense shedding of cell membranes behind the fast locomoting human melanoma HTB-140 cells.

Since Fedier and Keller [11] demonstrated in short lasting experiments that hypertonic solutions within 30 minutes inhibited movement of Walker murine carcinosarcoma cells, we examined the long lasting effects of these solutions on movement of these cells. Also in our experiments the Walker cells did not move not only in sorbitol but also in saccharose-supplemented hypertonic solutions and this inhibition persisted for hours. These cells reacted therefore differently than HTB-140 cells under similar experimental conditions. Nevertheless, when calcium concentration was increased by 3 mM CaCl_2 in these hypertonic solutions, the Walker cells produced pseudopodia and migrated (Tab. 5). The above described effects of hypertonic solutions on motile activities of human melanoma HTB-140 and murine carcinosarcoma Walker cells had no influence on cell viability.

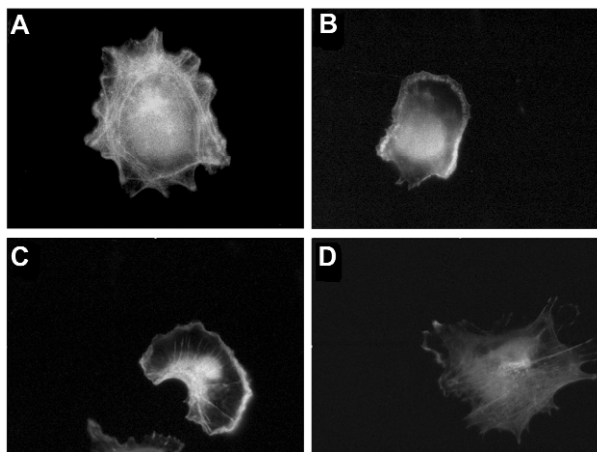


Fig. 5. Distribution of F-actin filaments in HTB-140 melanoma cells in hypertonic solution. Cells fixed in 3.7% paraformaldehyde and permeabilized with 0.1% Triton X-100 were stained with TRITC-phalloidin (500 ng/ml). Pictures were taken under a Leica fluorescent microscope (Leica DM IRE2) equipped with a Leica DC350 FX digital camera, objective 40x. A – cells in control conditions, B – cells in hypertonic solution of 9.5% saccharose (w/w) dissolved in DMEM (650 mOsm), C – cells in hypertonic saccharose containing medium after 2 h of incubation, D – cells in control medium after 3 h of incubation (with earlier 3 h pre-incubation in hypertonic conditions).

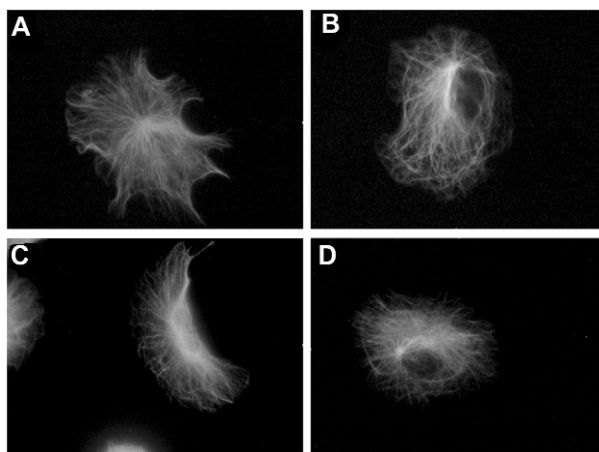


Fig. 6. Distribution of microtubules in HTB-140 melanoma cells in hypertonic solution. Cells fixed in methanol, blocked with 3% bovine albumin in TBA were incubated with monoclonal anti-human microtubule antibody (1:500) and anti-mouse IgG antibody conjugated to TRITC (Sigma). Pictures were taken under a Leica fluorescent microscope (Leica DM IRE2) equipped with a Leica DC350 FX digital camera, objective 40x. A – cells in control conditions, B – cells in hypertonic solution of 9.5% saccharose (w/w) dissolved in DMEM (650 mOsm), C – cells in hypertonic solution after 2 h of incubation in saccharose supplemented DMEM, D – cells in control medium after 3 h of incubation (with earlier 3 h pre-incubation in hypertonic conditions).

Tab. 5. Percentage of Walker carcinosarcoma 256 cells restoring their ability to migrate after altered periods of incubation under different hypertonic conditions.

Solution	Time of incubation [h]			
	1	2	4	6
5% sorbitol in RPMI	0	4	30	45
5% sorbitol + 3 mM CaCl ₂ in RPMI	10	32	46	73
9.5% saccharose in RPMI	0	0	7	12
9.5% saccharose + 3 mM CaCl ₂ in RPMI	0	13	41	59

DISCUSSION

The results presented in this communication show that the interpretation of results concerning the effects of hypertonic solutions on eukaryotic cells requires the following considerations: i) the cell responses to hypertonicity are time-dependent and, in at least some cases, cells after a shock response can adapt and show normal performance of active cell movement in strongly hypertonic conditions; ii) the cell responses to hypertonic solutions are dependent on the type of solute due to an interaction of solutes with cell membranes besides pure “osmotic” effects related to partial dehydration of cells, iii) cell motility changes

in the same hypertonic solution are dependent on cell type and can differ in different cells.

Animal cells are known to survive extensive dehydration [1, 44, 45]. The cell membrane is relatively permeable to water and cells behave as osmometers [1, 46]. The relative intracellular osmolarity in *Dictyostelium discoideum* cells growing in control conditions was found to equal 115 but increased up to 330 mOsm within 60 minutes in hypertonic solution containing 400 mM sorbitol added to Soerensen phosphate buffer and of 434 mOsm osmolarity. To equalize intracellular osmotic pressure with that of external hypertonic solution, cells can shrink osmotically, decreasing their volume, and accumulate osmotically active substances usually different from the osmolyte used in the experiment [46-48]. In our experiments none of the relatively strong hypertonic solutions investigated (with osmolarity in the range to 750 mOsm) had an influence on cell survival. All the observed effects on cell activities and morphology, including cell volume and cytoskeletal architecture, were found to be reversible on return of the cells to control culture conditions, as reported by others. For example, Schuster *et al.* [49] found that *D. discoideum* cells suspended in high osmolarity medium (400 mM sorbitol) rapidly reduced their volume by 50% and this was maintained as long as those osmotic conditions prevailed; but when re-diluted they re-established normal volume and movement within a few minutes. The *D. discoideum* cells in their soil habitat are often exposed to rapidly changing osmotic conditions and according to Steck *et al.* [50] they adapt to changes of medium tonicity mainly via the action of the contractile vacuole. Metazoan cells can also adapt to hypertonic solutions. Guthrie *et al.* [51] reported that bovine spermatozoa behaved as osmometers, survived and tolerated significant dehydration in 1 M solutions (up to 1200 mOsm) of such osmoprotectants as glycerol, dimethyl sulfoxide and ethylene glycol. These osmoprotectants when applied in 1 M hypertonic solutions caused different degrees of motile inhibition (6 to 90%) in bull spermatozoa. Schachtschabel and Foley [12] succeeded in the serial cultivation of Ehrlich ascites tumor cells in media of doubled osmolarity; and Clegg *et al.* [45] cultured mouse fibroblast-like L-929 cells in medium supplemented with 0.3 M sorbitol, that doubled the normal external osmotic pressure. Our observations confirmed that instant responses of cells to at least some of the hypertonic solutions involve inhibition of cell movements, as reported by Fedier and Keller [11]. Nevertheless, Walker carcinosarcoma cells, though originally inhibited in movement in hypertonic sorbitol solution, regained the capacity to move within hours of the incubation, or after the addition of calcium chloride to increase the calcium ion concentration. A similar temporal inhibition of movement was observed in *D. discoideum* amoebae. The cells, though still osmotically shrunk, actively moved when incubated for 8 hours in 10% sorbitol (w/w) dissolved in Chalkley's medium (628.25 mOsm). It therefore appears that in all cases when the hypertonic solution induced inhibition of cell movement the cells could

resume active locomotion after calcium ion concentration was increased in the sodium chloride, sorbitol or saccharose hypertonic solutions.

The effects of hypertonic solutions on cell motile activity were found to depend on the type of the solute besides the solution osmolarity. Thus the composition of the solution, the type of cells studied, and the time of cell exposure to hypertonicity were very important besides the water activity, i.e. osmolarity (osmotic pressure) of these solutions. Amoebae of *D. discoideum* were inhibited in their locomotion by 2% NaCl solution (670.5 mOsm), but significantly accelerated their movement in 2% KCl solution (587.5 mOsm) in spite of the fact that in both these hypertonic solutions the cells showed similar osmotic shrinkage. High potassium ion concentrations in medium are known to depolarize cell membranes in many cell types. Nevertheless, the BSS medium commonly used to culture *Dictyostelium discoideum* contains as monovalent ions mainly potassium, similarly as media used to grow plant, fungi and many invertebrates' cells [22], and as shown by Van Duijn *et al.* [37] these amoebae can locomote and show chemotaxis when their membrane is depolarized. What is more, on addition of calcium ions to 2% NaCl solution the cells restored movement though the osmolarity of the medium further increased and they did not regain their original cell volume. These observations can be explained by the known differences in the effects of sodium and potassium on proteins and membranes and their modulation by calcium ions [36, 52]. It seems interesting that Thiel *et al.* [53] came to the conclusion that an increase in the concentration of sodium rather than hypertonicity itself was responsible for the observed up-regulation of β -2 integrins in human polymorphonuclear leucocytes. The experiments carried out with human melanoma HTB-140 cells confirmed the motile observations carried out on *D. discoideum* amoebae in hypertonic solutions, supporting the conclusion that the type of solute has a great influence on cell motile responses to hypertonicity besides medium osmolarity.

Melanoma HTB-140 cells reacted differently to saccharose (650 mOsm) and sorbitol (630 mOsm) in DMEM hypertonic solutions than Walker carcinosarcoma cells. The differences in cell responses to hypertonic solutions when various carbohydrates are used as solutes can be explained by differences in cell membrane properties of various cells and by the known, though often overlooked, differences in the effects of different carbohydrates on cell membranes, liposomes, and phospholipid membranes [44, 54]. This conclusion is further supported by the observation that an increase in extracellular calcium caused restoration of movement inhibited in sorbitol hypertonic solution in the Walker carcinosarcoma cells.

The reported significant increase in the rate of human melanoma HTB-140 cancer cell movement in the hypertonic solution of saccharose under *in vitro* conditions should not be neglected when such solutions are applied in patients. Hypertonic solutions *in vivo* can perhaps stimulate movement of at least some cancer cells, which may be responsible for the spread of tumor cells. The activation of cell movement is dependent not only on gene activation (i.e. inside-

out signaling as demonstrated by Wyckoff *et al.* [16, 18, 19, 55]), but also on local environmental conditions, including medium osmolarity and solute present (i.e. outside-in signaling).

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