# Influence of Mono- and Multivalent Cations on the Electrokinetic Properties of Normal Human Lymphoid and Burkitt Lymphoma Cells

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*Summary.* Various mono- and multivalent cations, at constant ionic strength, affected the surface electrokinetic properties of normal human lymphoid and Burkitt lymphoma cells in a manner which reflected more the physicochemical properties and binding affinities of the cation than its valence. The effect was expressed in the changes of the magnitude of the net surface charge and in the shift of the isoelectric point of the surface. These changes were greater at the surface of Burkitt's lymphoma cells than at the surface of their normal cell-line counterparts.

The presence or absence of a particular macromolecule or ion in the ambient environment of a cell can result in changes in the cell surface properties and, consequently, in changes in cell function and behavior[2,3]. In particular, the type and/or valence of cations in the cell environment can influence the magnitude of the cell surface potential and, thereby, influence the course of various cellular interactions[4,5].

The aim of the present study was to investigate the effects of cations implicated in abnormal development, oncogenesis, and air and water pollution on the surface properties of normal lymphoid and Burkitt lymphoma cells at constant ionic strength.

*Materials and methods.* Normal human lymphoid cells (line $EBV_{25}$) and Burkitt lymphoma cells (line $EB_3$) were grown as suspension cultures in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics. The cells were prepared for electrophoresis by washing, with centrifugation, in 0.145 *M* NaCl and in one of the experimental solutions at pH 7.0, followed by resuspension in the experimental solution at the desired pH value. The electrophoretic mobility (EM) was measured as described previously[5]. The suspending media for EM measurements were chloride salt solutions of mono- or multivalent cations at an ionic strength of $3\times10^{-4}$ (or as indicated), prepared in 4 × glass distilled water, and supplemented with sucrose to maintain the osmotic pressure constant. The pH of the solutions was adjusted with 1 *N* HCl or 1 *N* NaOH. Cell viability was determined by the Nigrosin exclusion test[6] and electrophoretically[5].

*Results.* The amount of EM suppression exerted by the different cations increased, in general, with an increase in the valence of the cation (Table). Individual cations, however, displayed effects that deviated from the results predicted by the lyophobic colloid theory[4]. For example, although similar electrokinetic patterns were obtained with $Na^+$, $K^+$, and $Li^+$, (Figure a and b) and $Li^+$ caused essentially the same amount of surface charge suppression on both cell types, the magnitude of suppression exerted by $Li^+$ was more similar to that exhibited by some multivalent rather than by other monovalent cations (Table). On the other hand, $Cd^{++}$ and $Pb^{++}$ interacted with surface ionogenic sites differently than did $Ca^{++}$ and $Zn^{++}$, as indicated by the shape of their pH-EM curves (Figure a and b). In addition, $Cd^{++}$ and $Pb^{++}$ indicated an affinity for ionogenic sites with pK values between 4 and 5 at the surface of Burkitt lymphoma cells, whereas little or no interaction occurred, within the same pH range, at the surface of normal lymphoid cells. The similarity in the

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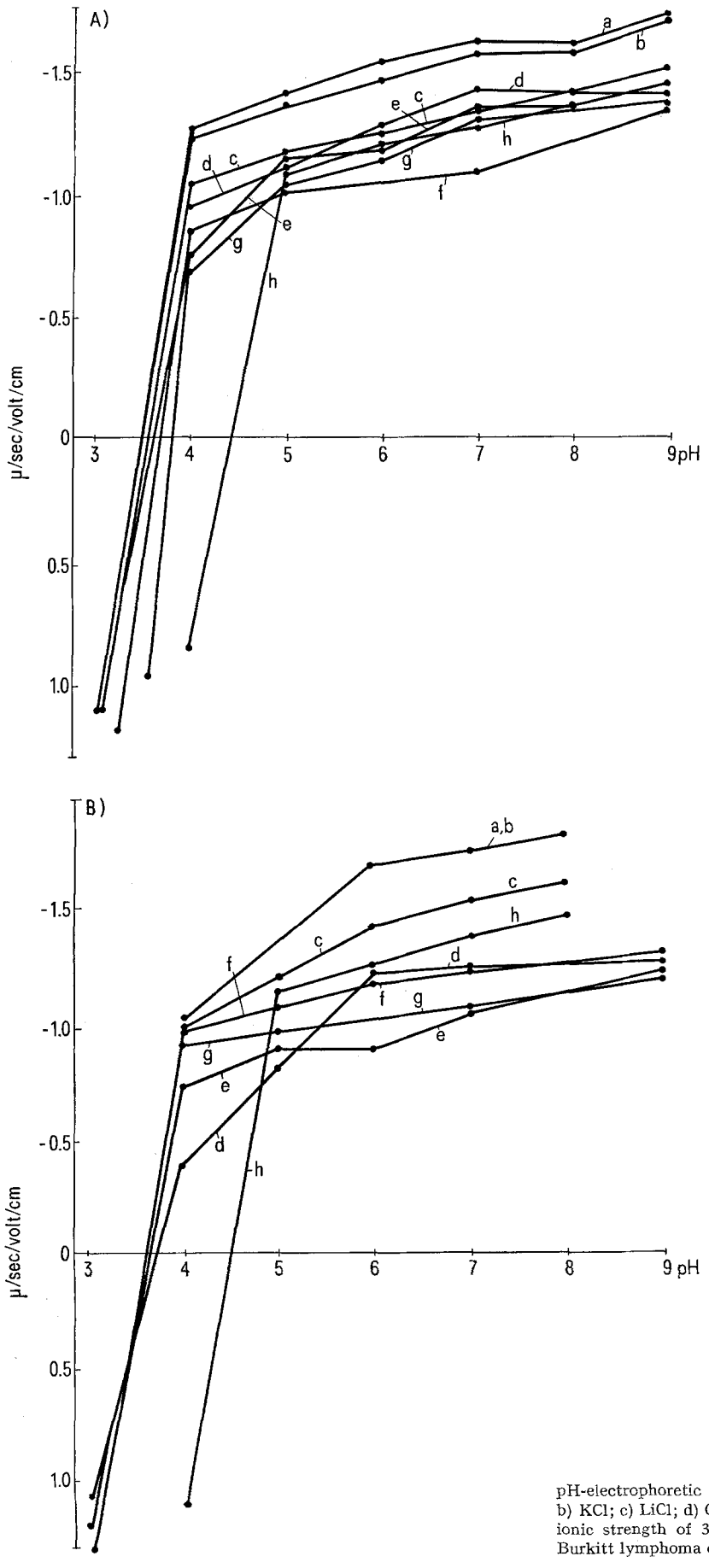
Effect of mono- and multivalent cations on the electrophoretic mobility of normal human lymphoid and Burkitt lymphoma cells at pH 7.0 (ionic strength = $3\times10^{-4}$, except where indicated)

| Electrolyte | EM (μ/sec/V/cm) | | Isoelectric point (pH) | | Suppression EM (%)[a] | |
|---|---|---|---|---|---|---|
| | Normal lymphoid cells | Burkitt lymphoma cells | Normal lymphoid cells | Burkitt lymphoma cells | Normal lymphoid cells | Burkitt lymphoma cells |
| NaCl ($1.45\times10^{-1}$)[b] | 0.941 | 1.38 | No I.P. | 3.5 | — | — |
| NaCl | 1.60 | 1.75 | 3.5 | 3.5 | — | — |
| KCl | 1.55 | 1.80 | 3.5 | 3.5 | 3.1 | (−2.9) |
| LiCl | 1.30 | 1.41 | 3.5 | 3.5 | 18.8 | 19.4 |
| LiCl ($1.45\times10^{-4}$)[b] | 1.14 | — | 3.0 | — | 28.8 | — |
| $CaCl_2$ | 1.41 | 1.24 | 3.5 | 3.5 | 11.9 | 29.1 |
| $CdCl_2$ | 1.32 | 1.06 | 3.5 | 3.5 | 17.5 | 39.4 |
| $PbCl_2$ | 1.31 | 1.08 | 3.8 | 3.5 | 18.1 | 38.3 |
| $ZnCl_2$ | 1.08 | 1.23 | 3.7 | 3.5 | 32.5 | 29.7 |
| $LaCl_3$ | 1.21 | 1.08 | 3.9 | 4.5 | 24.4 | 38.3 |
| $AlCl_3$ | 1.24 | 1.37 | 4.3 | 4.5 | 22.5 | 21.7 |
| $ThCl_4$ | 1.43 | 1.48 | 3.6 | 4.5 | 10.6 | 15.4 |

[a] Relative to EM in the presence of NaCl at an ionic strength of $3\times10^{-4}$. [b] Ionic strength.




pH-electrophoretic mobility relations in the presence of a) NaCl; b) KCl; c) LiCl; d) $CaCl_2$; e) $CdCl_2$; f) $Zncl_2$; g) $PbCl_2$; h) $AlCl_3$ at an ionic strength of $3 \times 10^{-4}$. A) Normal human lymphoid cells. B) Burkitt lymphoma cells.

electrokinetic patterns and in the amount of EM suppression of both cell types in the presence of $Zn^{++}$ suggested an affinity of this cation for ionogenic sites available on both surfaces. These sites, however, were probably different from the sites interacting with $Pb^{++}$ and $Cd^{++}$, at pH 4 to 5, as suggested by the decrease in EM of both cell types in the presence of $Zn^{++}$ and the relative increase in mobility exhibited by the normal lymphoid as compared to the Burkitt lymphoma cells in the presence of $Pb^{++}$ and $Cd^{++}$. This difference in the surface ionogenic site composition of the two cell types was further suggested by the difference in the electrokinetic patterns obtained in the presence of $Ca^{++}$: little or no $Ca^{++}$ was bound between pH 4 to 6 by Burkitt lymphoma cells, as indicated by the steepness of the slope; the flatness of the slope within the same pH range with the normal lymphoid cells suggests quantitative or qualitative differences in the surface ionic composition. The difference in the interaction of the cations with the surfaces of the two cell types was further shown by the amount of EM suppression caused, at pH 7, by $Ca^{++}$, $Cd^{++}$, $Pb^{++}$, $La^{+3}$, and $Th^{+4}$; in all cases, the suppression in EM of the Burkitt lymphoma cells was significantly greater (Table).

The tri- and tetravalent cations studied caused a significant shift of the isoelectric point at the surface to a higher pH, but their ability to suppress EM was less than that of some divalent cations (Table).

The results of the viability tests indicated 95% cell viability. Measurements of the electrophoretic mobility of the same cells suspended in one of the experimental solutions at pH 9.0, followed by measurements at pH 7.0[5], indicated that the effects exerted by the cations studied were reversible.

*Discussion.* These data demonstrated that, when the ionic strength of the medium was maintained constant, the effectiveness of mono- and multivalent cations to suppress the expression of surface ionogenic groups was not the same, which is contrary to the predictions made by the lyophobic colloid theory[4,5]. The effectiveness of the cations studied to suppress the EM appears to be related to the specific physicochemical properties of the cation, e.g., large hydrated ionic radius ($Li^{+}$), ability to form polymeric hydroxide complexes ($Al^{+++}$), differential affinity for available ionogenic sites at the surface ($Cd^{++}$, $Pb^{++}$, $Zn^{++}$, $Ca^{++}$). Changes in the cationic composition of the environment, even when the ionic strength remains constant, can, therefore, result in significant and unexpected changes in the expression of surface ionogenic sites. The influence of such changes on cell function and behavior can be of extreme importance, especially as some of the cations studied have been implicated in abnormal development ($Li^{+}$)[7], oncogenesis ($Cd^{++}$, $Zn^{++}$)[8,9] and air and water pollution ($Pb^{++}$, $Cd^{++}$, $Zn^{++}$)[10].

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## Age and Peritoneal Fluid Cellular Distribution in Women 20-40 Years of Age

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*Summary.* Cytologic aspiration specimens of peritoneal fluid revealed that mesothelial cell proportions were significantly reduced 19.2% in women between 26 and 35 years of age. Possibly, mesothelial cell renewal was decreased in women of the older age groups.

Cellular peritoneal fluid may provide a useful tool for studying age particularly in women. In previous studies we have defined an average cellular standard for peritoneal fluid in women and have stressed the possibility that cellular samples obtained from the Douglas pouch provide an index for understanding the normal cellular response within the pelvic cavity[2-6]. We observed in a few women under 20 years of age an elevated mesothelial cell count. In mice, age and sex difference profoundly influenced the cellular distribution within the abdominal cavity. Mesothelial cell proportionals increased from birth to sexual maturity in female mice but not in males[7,8]. Lymphocytes also, increased with advancing age from birth but the 'daisy cell' was seen only at weaning. The present study attempts to investigate the influence of age on peritoneal fluid cellular content of women between 20 and 40 years of age giving special attention to possible changes in the relative number of mesothelial cells.

Cul-de-sac aspirations were performed on 70 women between the ages of 20 and 40 years whose history and physical examination indicated that they were free of medical disorders. All women had normal menstrual cycles and were arranged into 4 age groups: 20–25, 26–30, 31–35 and 36–40 years. Group sizes ranged from 11 to 32 women. The posterior fornix of the vagina was thoroughly cleansed with a disposable cotton-tipped applicator and a 21-gauge needle with an accompanying stylet was used to enter the cul-de-sac. We immediately placed the aspirated specimen on an albumin-coated slide which was fixed and stained by Papanicolaou's procedure[9]. 200

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