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Red cell filterability determined using the cell transit time analyzer (CTTA): effects of ATP depletion and changes in calcium concentration

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Cell transit time analysis (CTTA) is a new filtrometric technique for assessing red blood cell deformability by measuring the conductivity change caused by passage of erythrocytes through a polycarbonate filter. Most reported studies to date using CTTA have focused on the transit time (TT), the duration of passage of an individual red cell through a micropore. Bulk flow rate has not been previously measured via CTTA. The use of new enzyme based cleaning solutions make it possible to reduce clogging in micropore filters. Therefore, valid measures of the number of red cell transits per unit time (counts/s; C/S) can now be obtained. We evaluated both parameters, TT and C/S, as indicators of red cell filterability. Our goal was to evaluate the effect of metabolic changes shown by alternative techniques to affect red cell deformability. The two best established factors are changes in intracellular [ATP] and [Ca^{2+}]. ATP depletion produces a very small increase in TT but a very marked decrease in C/S. In contrast, the addition of low concentrations of calcium produces an increase in TT with minimal decrease in C/S. The effects of calcium appear to be complex. The substantial changes in intracellular calcium induced by the ionophore A23187 result in a curvilinear pattern of increase in transit times and reduction in counts per s. Lanthanum, which inhibits egress of intracellular calcium, causes an increase in TT with a drop in C/S. We conclude that CTTA demonstrates the same changes in red cell deformability measurable by alternative filtrometric techniques; however, CTTA furnishes two separate and independent parameters which may be used to evaluate red cell deformability.

Introduction

Cell transit time analysis (CTTA) is a new filtrometric technique for assessing red blood cell (RBC) deformability by measurement of the conductivity change caused by the passage of individual cells through a polycarbonate filter [1,2]. Occupancy of a filter pore decreases the conductivity, denoting a transit event. The duration of the conductivity change reflects the time required for the RBC to traverse the length of the pore (transit time (TT)). Passage of red cell doublets, platelets or white cells result in conductivity changes and, hence, transit times (TT) very different from those of single red cells, permitting accurate identification. CTTA has been used to demonstrate decreased transit times of aged versus young red blood cells, dinitrophenyl treated red cells and heat treated red cells [3]. Red cells from poorly controlled diabetic patients have been shown to have longer transit times than those from non-diabetic controls [4,5].

These CTTA results, while intriguing, have not been directly compared to data produced using previous filtrometric techniques, which generally measure the bulk flow rate of red cells through the filter [6,7]. In fact, CTTA does permit the determination of RBC flow, given as the number of transit events per unit time (counts/s; C/S). However, this parameter has not been reported in CTTA work published to date; the lack of these data can be ascribed to two factors. First, there has been greater interest in the unique capability

Abbreviations: CTTA, Cell transit time analysis; C/S, counts per s; RBC, red blood cell.

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of CTTA to determine transit times. Second, there is the implicit assumption that measurement of C/S would be invalidated by the rapid clogging of pores which affects all filtrometric procedures. Although filters may be cleaned with detergents and bleach between assays, the success of these agents in reopening clogged pores is variable. In contrast, transit times are unaffected by clogging since they are determined solely in patent pores.

Recently, powerful new enzymatic cleaning agents have been introduced to obviate clogging in clinical laboratory hematology and chemistry analyzers. We have found that these agents are much more effective than detergents and bleach in maintaining patency of filter pores. We have, therefore, carried out cell transit time analysis, quantitating not only TT but C/S, in the hope of obtaining results more easily compared to the flow rates measured by previous, alternative filtrometric techniques.

We have used this approach to assess metabolic influences on red cell deformability, an area not previously studied via CTTA. Our goal was to evaluate the effect of metabolic changes shown by alternative techniques to affect red cell deformability. The two best established metabolic factors are changes in intracellular [ATP] and [Ca^{2+}]. Intracellular depletion of ATP renders red cells more rigid [8,9]. Red cell calcium accumulation markedly decreases red cell deformability [8–13].

Methods

Heparinized blood samples were spun at 750 × *g* for 10 min. Red cells were then separated and washed once in isotonic Hepes buffer (25 mM Hepes, 113 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 5 mM glucose (pH 7.4), 295 mosmol/l). A 50% dilution of packed RBCs was made in this buffer, with appropriate addition of various agents in given experiments, and incubated for one hour at 37 °C. Mean corpuscular volumes were determined by dividing the spun hematocrit by the cell count. A 1 to 1000 dilution of the incubated red cell suspension was then made in the same buffer, usually with a final assay [RBC] of 1000–5000 RBC/mm^3. The mean corpuscular volume was not affected by dilution in this buffer.

Cell transit time analyzer red cell measurements. This device has been extensively described elsewhere [1–3]. Polycarbonate filters containing 30 centrally located non-overlapping pores are used; pore diameter was 5 microns and the length of the cylindrical micropores was 15 microns. The filter is horizontally mounted, one surface exposed to buffer and RBCs in the sample chamber, the other surface to buffer in a receiving chamber. The hydrostatic pressure gradient is established by adjusting the height of buffer in the respective chambers. Conductivity, reported in arbitrary units called the polarization, is measured across the CTTA filter by a voltmeter. Occupancy of a pore by a single cell reduces the conductivity across that pore, causing a downward deflection in the polarization. The baseline polarization is set at 500–525 at the beginning of each assay. Deflections in the polarization from baseline are recorded as transit events. The duration of each deflection is computed to give the transit time (TT). The CTTA system provides an analog digital readout of the polarization, as well as cumulative transits (counts), counts/s (C/S), and statistical information on the transit times, including mean value and standard deviation. The assay is run to completion of a preset number of total counts. At the end of the assay, results, including a frequency histogram of transit times, are computed.

Samples were run on the CTTA at 2 cm H_2O pressure at 37 °C. Each sample was run within 2 h of preparation. Each assay consisted of determination of 1200 red cell transits per histogram. Filters were sonicated between individual assay runs in 2-fold concentrated Hemazyme (Hematronix, Benicia, CA). This solution of proteolytic enzymes and surfactants removes cellular debris which might otherwise clog individual pores. The filters were then subsequently sonicated in 0.1% Triton X-100, and then distilled water to remove all traces of Hemazyme. Each filter is generally usable for about 100 assays. Using Hemazyme treatment, we usually found no decrease in polarization or C/S over the course of the assay, as well as no systematic drop in C/S after repeated assays. When such a decrease occurred, as a rule in a filter nearing its usable life expectancy, we concluded that pores were clogged, thus invalidating the results. We would then subject the filter to prolonged sonication (up to 5 min) in Hemazyme. Usually, such treatment was successful in restoring filter function.

The red cell transit counts/s were divided by the red cell concentration in the sample to obtain a normalized value. In calculating the red cell mean TT, we focused on the major transit peak, thus eliminating TTs that were scattered outside the 99% confidence limits of the peak, i.e., greater than 3 S.D. from the mode. This was done since inclusion of outlying transit events with very long TTs would disproportionately influence the observed mean. The proportion of outlying transit events was monitored to ensure that no systematic effect of biochemical modifications on a small fraction of the cells would be overlooked. The CTTA software outputs a parameter called the efficacy which numerically reflects the proportion of outlier cells. Filters are manufactured with a coefficient of variation of pore size within 2%. However, we have found interfilter variation of up to 20%, particularly between different filter batches. For this reason, each

individual experiment shown, including all duplicates, was run with a single filter.

Statistical Methods. TTs, MCVs and C/S were compared using standard ANOVA techniques. Ratios were compared using non-parametric ANOVA procedures (Kruskal-Wallis, Friedman ANOVA, Mann-Whitney, Wilcoxon signed ranks) [14]. Standard polynomial curve fitting procedures were used. All curves were drawn using polynomial fitting procedures to produce an unbiased estimate of the shape of the curve.

Results

Reproducibility of TT versus C / S

Using a single blood sample within a single assay on one filter, the coefficient of variation (c.v.) for TT was 4% and for C/S 11% for 16 repetitions. Using a single filter for a single individual giving blood samples over four months, c.v. for TT was 5% and 14% for C/S. Using a single sample in an assay using 21 different filters from a single batch, c.v. for TT was 7% and 15% for C/S.

TT and C / S as a function of assay time

Two factors could potentially affect these two experimental parameters. One is differential sedimentation of the red cell suspension. However, the time-course of our assay is usually 1 min or less to reach 1200 cell transits, too fast for sedimentation to have a significant effect. The second factor is clogging of the filter, which occurs progressively in all filtrometric procedures. We demonstrated that the time-course of our assay is too rapid to be affected by either sedimentation or clogging by running the assay far beyond the usual time-course, until significant clogging occurred (Fig. 1). At

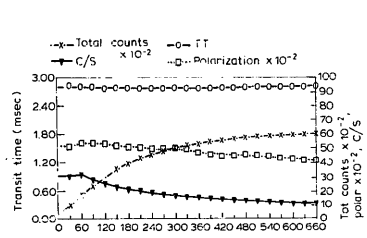

Fig. 1. Transit times, counts/s, total counts, and polarization as a function of time. We ran a sample far beyond the usual accumulation limit of our assay (1200 total counts). This was done to demonstrate clogging of a filter. Transit time remained invariant throughout the assay. Total counts increased linearly for the first 1800 cells, then slope of increase (C/S) began a progressive slow fall. Polarization was stable for the first 1800 cells, then began a slow fall.

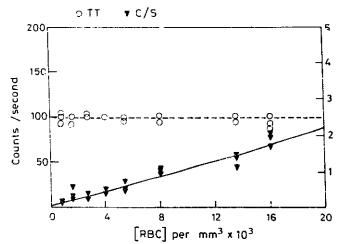

Fig. 2. Transit times and counts/s as a function of red cell concentration in sample chamber. Transit times, plotted against the right axis, and total C/S, plotted against the right axis were determined for the given red cell concentrations. There was no change in TT over the above range. Total C/S increased linearly.

an initial rate of 30 counts/s, the usual assay terminates within 40 s. We show that total counts increased linearly for 100 s. After this point, there was a slow decline in C/S. By 660 s, C/S had reached a rate of 10 counts/s, one third of the initial rate. Polarization also began to decrease at about 100 s. The decrease in polarization was not as great as in C/S since even clogged pores do retain some conductivity. Transit time is calculated purely on the basis of those pores which remain available to red cell passage and was, therefore, invariant over the 11 min shown. The important point is that over the usual 1200 cell accumulation time of our assay, C/S, polarization and TT are invariant, thus signifying that clogging, sedimentation and any other factors which could potentially affect our results do not play a role. When we do see a decrease in C/S or in polarization, this is interpreted as a clogged filter. It is possible by sonication and Hemazyme treatment to recover even a filter as drastically treated as in this 11 min run.

TT and C / S as a function of red cell concentration

For TT and C/S to be reliable filtrometric measurements, they must be relatively invariant as a function of [RBC] in the sample. Total C/S is, of course, dependent on the red cell concentration. Within our usual experimental range (1:1000 dilution of packed cells ($(1-5)\cdot 10^3$ RBC/mm^3)), total C/S increased linearly with [RBC] (Fig. 2), thus resulting in an invariant normalized C/S, measured as the slope. TT was invariant over this range. At [RBC] 10 to 20-fold higher than our experimental range, there was an apparent saturation phenomenon in C/S (Fig. 3). There was also an apparent decrease in TT. At $150\cdot 10^3$ RBC/mm^3, baseline polarization decreased over the time-course of an individual assay, signifying clogging of pores.

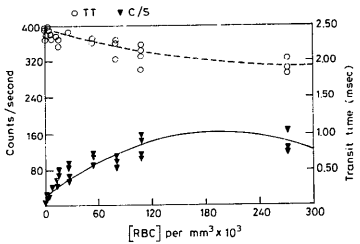

Fig. 3. Transit times and counts/s as a function of red cell concentration over a broad range. Transit times, plotted against the right axis, and total C/S, plotted against the right axis were determined for the given red cell concentrations.

Effect of cell size on TT and C/S

Cell size change, created by osmotic modification, appears to exert a minimal effect on transit times (1,5). We reconfirmed this prior work in order to also assess the effect of osmolar changes on C/S. As previously demonstrated, TT were essentially unaffected in a broad range (250–350 mosm/l) around isotonicity, despite a 20% overall change in red cell size (Fig. 4). Only at more extreme hypotonicity (< 225 mosm/l) or hypertonicity (> 400 mosm/l) was there an effect on TT. As previously shown; despite a marked reduction in cell size at hypertonic levels (450 mosm/l), TTs actually increased. C/S was relatively invariant in a range of 250–350 mosm/l. However, at extreme hypotonic or hypertonic levels, there was a relatively sharp dropoff in C/S. Thus, despite a 20% drop in MCV, cells in hypertonic medium showed both a small increase in TT and a substantial decrease in C/S.

Effect of ATP depletion

Red cells were incubated with gentle stirring for 24 h, at 37 °C or at 0 °C, with or without the addition of 5 mM iodoacetamide, which prevents endogenous formation of ATP. We compared CTTA results for these 24 h incubated cells to fresh cells, incubated for 1 h at 37 °C with and without exposure to iodoacetamide (Fig. 5). The effect on TTs was very small. Only the 37 °C incubated, iodacetamide treated cells showed an increase in TT, with that increase only on the order of 5%. The small magnitude of this increase was somewhat surprising in view of the pronounced echinocytic transformation which occurs in ATP depleted cells [8,9]. There was no significant change in MCV in these incubated cells, but we verified microscopically a marked echinocytic transformation. In contrast to the small change in TTs, there was a 60% decrease in C/S in 37 °C incubated red cells in the absence of iodoacet-

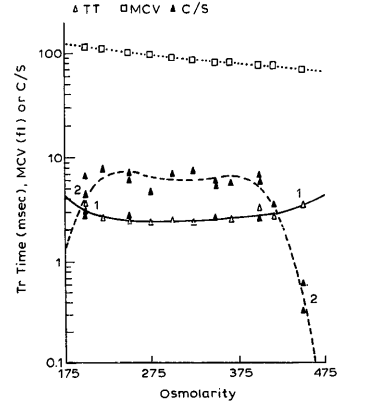

Fig. 4. Variation in red cell transit times, counts per s, and mean corpuscular volume as a function of osmolarity. Red cells were suspended in Hepes buffer (25 mM Hepes, 5 mM KCl, 0.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.1 mM ATP, 5 mM glucose, with NaCl concentration varied to achieve desired osmolarity, pH 7.4). Transit times, C/S and MCV, in their respective units, are plotted on a logarithmic ordinate as a function of buffer osmolarity (mosm/l). MCV progressively decreased with increasing function of buffer osmolarity (mosm/l). MCV progressively decreased with increasing osmolarity. C/S and TT were essentially invariant over a broad range around isotonicity, only rising at extremes of 200 mosm/l and 400 mosm/l. $n = 5$; $^1P < 0.05$. $^2P < 0.01$.

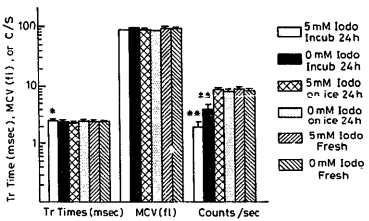

Fig. 5. The effect of ATP depletion on transit times, MCV and counts per s. Red cells were incubated with gentle stirring either at 37 °C (Incub) or at 0 °C (on ice) for 24 h in Hepes buffer (25 mM Hepes, 113 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 5 mM glucose (pH 7.4), 295 mosmol/l) with or without the addition of 5 mM iodoacetamide (Iodo). TT, MCV and C/S were compared with results for cells freshly prepared and assayed in the same buffers after one hour incubation at 37 °C (Fresh). Cells kept on ice were resuspended in buffer at 37 °C, immediately prior to assay. Values given as mean ± S.E., $n = 10$; * $P < 0.05$; ** $P < 0.01$.

amide, and an 80% decrease in the presence of iodoacetamide (Fig. 5). A 1 h exposure to iodoacetamide at 37 °C resulted in no changes in TT or C/S.

Effect of adenine derivatives on fresh red cells

We added adenine derivatives to fresh cells in concentrations varying from 0 to 10 mM. Cells were incubated with the given derivative for 1 h at 37 °C. There was no effect of even very large changes in external concentration of ATP, ADP, AMP, adenosine, dibutyrl cAMP, 8-bromo cAMP or theophylline on TTs, C/S or MCV.

Effect of calcium

We incubated on fresh red cells at calcium concentrations ranging from 0 to 10 mM (Fig. 6). Results were expressed relative to the condition of 0 $[Ca^{2+}]$. There was no effect on MCV. At $[Ca^{2+}]$ of 1 mM and above, there was an increase in TT on the order of 10%. C/S was did not show a significant change from 0 to 5 mM $[Ca^{2+}]$. At 10 mM, there was a modest decrease in C/S. A similar experiment was performed, varying $[Mg^{2+}]$, with and without the presence of added calcium. There was no effect on TT or C/S.

Effect of calcium ionophore

Polynomial curvefitting of the increase in TT and decrease in C/S with increasing $[Ca^{2+}]$ suggested a curvilinear relationship. To further explore this possibility, we used the calcium ionophore A23187 to increase intracellular calcium levels. Calcium loading in the presence of A23187 is known to reduce red cell size, induce echinocytosis, and increase red cell rigidity

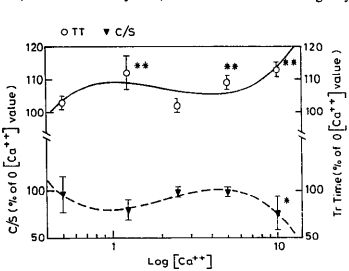

Fig. 6. The effect of varying external calcium concentration. Red cells were incubated at 37 °C for 1 h in Hepes buffer (25 mM Hepes, 113 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 5 mM glucose and varying concentrations of $CaCl_2$ (pH 7.4), 295 mosmol/l) and then run through the CTTA in the same buffer. Values for TT (top curve) and C/S (bottom curve) are given as percentage ratio to the value for the 0 $[Ca^{2+}]$baseline condition. MCV was invariant and is not presented. Values given as mean ± S.E., $n = 7$; * $P < 0.05$; ** $P < 0.01$.

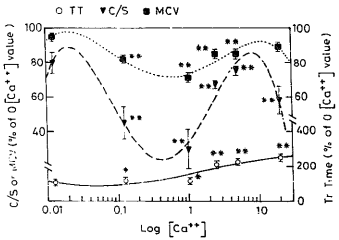

Fig. 7. The effect of varying external calcium concentration in presence of calcium ionophore. Red cells were incubated at 37°C for 1 h in varying concentrations of $CaCl_2$ (pH 7.4), 295 mosmol/l) in the presence of A23187 ($1.5 \cdot 10^{-6}$ M) and then run through the CTTA in the same buffer. Values for MCV (top curve) and C/S (middle curve), both plotted against the left (top) axis, and values for TT, plotted against the right (bottom) axis, are given as percentage ratio to the value for 0 $[Ca^{2+}]$baseline condition. Values given as mean ± S.E., $n = 8$. * $P < 0.05$; ** $P < 0.01$.

[11,12,15,16]. We varied $[Ca^{2+}]$ in the presence of $1.5 \cdot 10^{-6}$ M A23187 (Fig. 7). TT showed a small increase initially with increasing $[Ca^{2+}]$. Then, at about 1 mM $[Ca^{2+}]$, there was a sharp increase in TT of the order of 100% over the 0 $[Ca^{2+}]$ baseline. The effects on cell size and C/S as a function of $[Ca^{2+}]$ were very complex. There was an initial modest decrease in MCV, to about 80% of 0 $[Ca^{2+}]$ value. In accordance with our osmotic modification experiment, showing minimal effect of cell size on TT and C/S, C/S showed a substantial drop despite the decrease in cell size. However, there was a nadir in the decrease in both MCV and C/S at a $[Ca^{2+}]$ of about 1 mM, with a subsequent modest increase in cell size and a more marked increase in C/S, though not to baseline 0 $[Ca^{2+}]$ levels. Thus, despite an increase in cell size, C/S actually rose modestly with increase in $[Ca^{2+}]$ from 1 to 5 mM. This once again showed that cell size is not a major determinant of red cell deformability on CTTA.

Effect of lanthanum

Lanthanum at a concentration of 0.2 mM is known to block red cell Ca^{2+} extrusion by the Ca^{2+}-Mg^{2+} ATPase pump [17,18]. Physiological shear stress increases red cell Ca^{2+} permeability [19]. Vortexing cells for 5 s produces shear that is comparable to shearing with a cone-plate viscosimeter [20] and, in the presence of 0.2 mM LA^{3+}, causes a substantial increase in erythrocyte intracellular $[Ca^{2+}]$ [21]. We vortexed cells in the presence of varying $[Ca^{2+}]$ and La^{3+} (Fig. 8). There was an apparent decrease in C/S from the 0 Ca^{2+} condition with increasing $[Ca^{2+}]$; however, this change was confounded by substantial time progressive

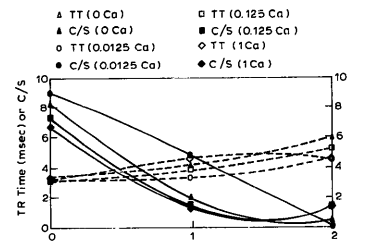

Fig. 8. Time-course of the effect of lanthanum. Red cells were incubated at 37 °C for 1 h in Hepes buffer (25 mM Hepes, 113 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 5 mM glucose and varying concentrations of $CaCl_2$ (pH 7.4), 295 mosmol/l) in the presence of 0.2 mM $LaCl_3$. 30 min into the incubation period, cells were vortexed for 5 s.

blockage of filter transit. This blockage was reflected by substantial increases in TT and decreases in C/S, occurring within an hour of preparing our assay samples. Cell free samples of buffer containing La^{3+} demonstrated progressive turbidity over this same time period. It was possible to remove this turbidity by filtering the solution through 0.2 micron pores. We concluded that, in our buffer, lanthanum formed a precipitate which occluded our filter pores. We were able to circumvent this problem by sedimenting our

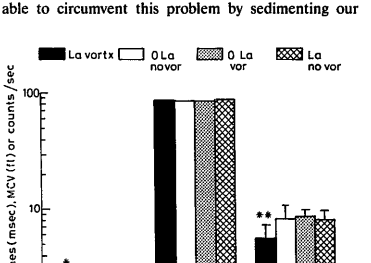

Fig. 9. The effect of lanthanum immediately added prior to assay. Red cells were incubated at 37 °C for 1 h in Hepes buffer (25 mM Hepes, 113 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 5 mM glucose and 2.5 mM $CaCl_2$ (pH 7.4) 295 mosmol/l in the presence of 0.25 mM $LaCl_3$. 30 min into the incubation period, cells were vortexed for 5 s. After incubation, cells were then sedimented by centrifugation and rapidly resuspended in fresh buffer, which had just been filtered through 0.22 micron Corning filters to remove the precipitate. The sample was then immediately run through the CTTA. Values given as mean ± S.E., $n = 6$; * $P < 0.05$; ** $P < 0.01$.

incubated samples by centrifugation, then resuspending in 0.2 mM lanthanum containing buffer, which had just been filtered through 0.22 micron Corning filters. Then we immediately ran the sample through CTTA. With this very laborious approach, we found, in the presence of 2.5 mM Ca^{2+} and 0.2 mM La^{3+}, and with vortexing, there was a small increase in TT and a substantial decrease in C/S (Fig. 9). There was no effect on cell size. In the absence of La^{3+}, or in the presence of La^{3+} with no vortexing, TT and C/S were no different from the condition of 2.5 mM Ca^{2+} alone. Furthermore, we were able to immediately reverse the induced changes in TT and C/S by filtering the red cells and resuspending them in a La^{3+} free medium.

Discussion

Cell transit time analysis is a new filtrometric technique which permits accurate measurement of duration of passage of an individual erythrocyte through a micropore. Our results confirm the effects of ATP depletion and change in $[Ca^{2+}]$ on red cell deformability, as previously demonstrated with other filtrometric techniques. As shown with alternative techniques, even supraphysiologic extracellular concentrations of adenine derivatives have minimal effect on erythrocyte deformability. Significant intracellular ATP depletion reduces deformability. Similarly even supraphysiologic extracellular $[Ca^{2+}]$ changes cause relatively minor effects. However, increasing intracellular $[Ca^{2+}]$ with calcium ionophore or by preventing egress of calcium by using La^{3+} significantly reduces deformability.

These data, more than simply confirming prior filtrometric results, suggest a potential advantage of CTTA as a rheologic technique. The transit phenomenon we measure is the passage of an erythrocyte through a filter pore. The complexity of this process has been extensively analyzed previously [22–25]. The process of pore transit begins with the erythrocyte first entering the pore. This entry phase requires that the cell must first impact on the pore. Then a very dramatic shape change must occur to allow the cell to initially enter the pore, analogous to the change to a 'bullet' or 'slipper' shape required for entry into small capillaries [26–28]. Once into the pore, having changed its shape, a different set of membrane events must then occur in order for the cell to pass along the length of the pore. These events represent more of a viscous deformation as the red cell membrane rotates around its fluid contents on its way through the pore [29,30].

Under the conditions we employ, the transit time is not rate limiting in the passage of cells across the pore. Typically, in our usual RBC concentration range, from 10–30 cells will transit the filter in a second, that is a rate of less than one cell per pore per second. Conversely, the transit time for each cell is on the order of

3 ms. Therefore, the overall duration of a single cell passage into and then through the filter is several orders of magnitude slower than TT. Thus, C/S, normalized for RBC concentration, is a measure of flow independent of TT. We postulate that TT is a measure of the velocity of red cell transit through the length of a pore, once having entered a pore and thus having undergone a marked shape change. Conversely, C/S presumably reflects other properties, including the probability of a red cell impinging on a pore and the ability of the cell, once having impacted the pore, to undergo the shape change necessary to enter the pore. Under the conditions we employ, C/S is invariant as a function of cell concentration. Therefore, the number of impacts on a given pore is presumably not rate limiting. Rather, it is the entry phase which appears to be rate limiting. The entry phase is presumably quite dependent on cell shape. Thus, in retrospect, it is not surprising that the pronounced echinocytic transformation of ATP depleted cells has little effect on TT, but results in a substantial drop in C/S. Others have previously called attention to the differential effect of ATP depletion and shape change on certain filtration properties but not on others [31].

Therefore, the potential advantage of CTTA over alternative filtrometric techniques [6,7] is to furnish two potentially different parameters of red cell deformability. Our data suggest that TT and C/S are affected separately and differently under certain conditions. Neither parameter appears to be highly dependent on red cell size since, in hypertonic conditions, despite decreasing cell volume, TT tends to increase and C/S to decrease. The lack of effect of cell size is not surprising. Reinhart and Chien have previously emphasized that cell size is not as important a determinant of erythrocyte pore transit as cell viscosity for small pore sizes [31]. The effect of ATP depletion is much more on C/S than on TT. Conversely, at low calcium concentrations, TT is modestly increased, with no effect on C/S. These data confirm other studies suggesting different mechanisms by which Ca^{2+} and ATP depletion affect red cell deformability [15]. However, the effects of calcium ionophore and lanthanum involve both TT and C/S. The relationships are very complex, as illustrated by the bimodal behavior of MCV and C/S with increasing $[Ca^{2+}]$ in the presence of ionophore. Once again, moderate changes of cell size induced by the ionophore treatment have a minimal effect on TT and C/S.

The basis for the differential effects on C/S and TT is not yet apparent. Clearly, morphological changes, such as induced by ATP depletion, impact much more on C/S than on TT. It has long been appreciated that various rheological procedures are more or less affected by red cell morphology [32]. We can speculate that the physicochemical effects of various agents on the mechanical deformations involved in cell entry are different from those required for passage once inside a pore. Cell transit time analysis provides a method for separate quantitation of these effects.

Acknowledgment

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