Studies of Cell Pellets: I. Electrical Properties and Porosity

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ABSTRACT Cell pellets formed by centrifugation provided a good system to study the osmotic behavior, electroporation, and interaction between cells. Rabbit erythrocyte pellets were used in this study because they were simpler than nucleated cells to model analytically. Structurally, cell pellets possessed properties of porous solid bodies and gels. Electrically, cell pellets were shown to behave as a parallel set of resistance, R_p, and capacitance, C_p. Information on pellet structures was obtained from electric measurements. The pellet resistance reflected the intercellular conductivity (porosity and gap conductivity), whereas the pellet capacitance depended mostly on membrane capacitance. The pellet resistance was more sensitive to experimental conditions. The intercellular gap distance can be derived from pellet porosity measurements, providing the cell volume and surface area were known. R_p increased and relaxed exponentially with time when centrifugation started and stopped; the cycles were reversible. When supernatants were exchanged with solutions containing hypotonic electrolytes or macromolecules (such as PEG) after the pellets were formed, complicated responses to different colloidal osmotic effects were observed. A transient decrease followed by a large increase of R_p was observed after the application of a porating electric pulse, as expected from a momentary membrane breakdown, followed by a limited colloidal-osmotic swelling of pelleted cells. The equilibrium values of R_p, C_p, pellet porosity, and intercellular distances were measured and calculated as functions of cell number, centrifugation force, and ionic strength of the exchanged supernatant. Thus, the structure and properties of cell pellets can be completely characterized by electrical measurements.

INTRODUCTION

Cell pelleting by centrifugation is a routine procedure in cell biology. The procedure is certainly traumatic for cells. Even at moderate accelerations, G, mechanical forces can deform cells and affect their morphology, forcing cell membranes to form tight contacts. However, even being brought in close contact, cell membranes stay apart because of the balance between external compressive forces and Van der Waal's attraction on the one hand, and different long- and short-ranged repulsive forces on the other. At large distances, the electrical forces are well described by the DLVO-theory (see, e.g., Ross and Morrison, 1988), whereas at smaller distances, entropic (Israelashvili and Wennestrom, 1991) or hydration (Parsegian and Rand, 1991) repulsive forces are dominant. For cell membranes, steric forces caused by the overlapping of membrane glycocalyx can also be important. The tightness of contact is an important consideration in cell interactions and recognition, as well as in cell adhesion and fusion (Wilschut and Hoekstra, 1991).

Specific properties of cell pellets derive from their porous structure. Although the physical properties of cell pellets have not been studied in detail, one may suppose that cell pellets behave differently from common porous or granular solid materials (Jaeger and Nagel, 1992), because cells cannot be modeled as either solids or liquids. Mammalian cells are usually very changeable in shape and volume. Hence, one might expect the cell pellet structure to be highly dependent on cell state and experimental conditions. Furthermore, cells in the pellet form can respond differently to external environment than if they are in suspension. For instance, tumor cells have different susceptibility to high energy electromagnetic shock waves in pellets than in suspensions (Smith et al., 1991).

Changes in the cell pellet structure can be followed by electrical measurements. It was found that both the fusion yield and the pellet electrical resistance (R_p) increase with G, and that different mammalian cells can be fused effectively in the pellet form. (Abidor et al., 1993). The few previous studies of cell pellets were performed with nucleated cells having a wide variety of physiological responses to various stimuli and conditions. The results are sometimes difficult to interpret. Mammalian erythrocytes lack nuclei and bulk cytoskeleton. Their behavior in pellets depends mainly upon the permeability of their plasma membranes, so that the results are easier to understand in physicochemical terms. In these two back-to-back papers, rabbit erythrocyte pellets were used as a model system for studying different phenomena related to cell pellet structures: osmotic properties, electroporation, electrofusion, electrohemolysis, and membrane interactions. This first paper aims to elucidate a relationship between the structure and electrical characteristics of cell pellets.

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MATERIALS AND METHODS

Cells and solutions

Erythrocytes obtained from fresh rabbit blood were washed twice and resuspended in isotonic (300 mOsm) PBS (150 mM NaCl + 5 mM NaPi; pH 7.3) to a concentration of $\sim 10^8$ cells/ml, as estimated by manual counting with a hematometer chamber under a microscope. In some experiments,

washed cells were incubated at 37°C for 30 min in PBS containing pronase E (1 mg/ml, Sigma Chemical Co., St. Louis, MO) and then washed with PBS.

All experiments were performed in PBS or sucrose-PBS buffers (pH 7.3) with different ionic strength (50–450 mM) or osmolarities (in the range of 200–1400 mOsm), as measured by means of a vapor pressure osmometer (Model 5130B, Wescor). Solutions of higher osmolarities were prepared by adding PEG (M_r 8000, Sigma) in isotonic PBS.

Experimental setup

Design of the experimental setup (Fig. 1) based on a bench-top centrifuge (IEC HN-S) was generally similar to that developed earlier for cell electrofusion (Abidor et al., 1993). The working rotor radius, i.e., the pellet-to-rotor axis distance was 15.5 cm (Fig. 1 *A*); thus, at the experimental speed of 2300 rpm, the acceleration G was ~900 × g. The centrifuge speed was precisely measured by a digital stroboscopic tachometer (Edmond Scientific, Barrington, NJ). The 1.5 ml capacity chambers (Fig. 1 *A*) consisted of two stainless steel electrodes screwed to a teflon body with a cone-shaped collector and a pellet hole (3-mm diameter, ~1 mm height). During centrifugation, cells were collected from the suspension into the hole, forming a compact pellet on the bottom electrode. Two coaxial brass contact discs, separated by a spacer-insulator, were fixed on the axle of the rotor. A pair of carbon brushes was fixed on the centrifuge cover to provide electric connections between the chamber and the external electrical circuit during centrifugation when the cover was closed.

A circuit used for electroporation and electrical measurements is shown schematically in Fig. 1 *B*. The chamber with a cell pellet is given here by an equivalent circuit consisting of the chamber resistance R_{ch} (corresponds to the chamber filled with a given cell suspension or solution), the pellet capacitance C_p, and the pellet resistance R_p. A variable loading resistor R_l was placed between the chamber and either a low-voltage generator (LVG) or in some experiments a high-voltage pulse generator (HVG). LVG (Model 3300, Precision Instrument) produced single or periodic rectangular bipolar test-signals (usually of ~500-mV amplitude at 10–50 μs duration), whereas the HVG gave single short (in the range of 10 μs to 1 ms) high-voltage (up to 1.5 kV) quasi-rectangular pulses. A signal U_o delivered by LVG or HVG and a voltage drop U_{ch} on the chamber were recorded and measured using a digital storage oscilloscope (Model OS-6121, Iwasu, Japan).

Procedures

Typically, the centrifuge chamber was filled with 1 ml of a suspension containing a required number of rabbit erythrocytes N_c and subjected to a constant acceleration G (900 × g unless otherwise noted) for at least 20 min to form a compact erythrocyte pellet. Usually pellets contained from 7.5 to 150 × 10^6 cells, which corresponded to 40–800 cell layers (at the average rabbit erythrocyte diameter of 7.3 μm (Ponder, 1971); a minimum of 0.185 × 10^6 cells is required to form a compact monolayer at the bottom of the 3-mm hole within the chamber). All electrical measurements were made within 20–30 min after subjecting the cells to a given centrifugal acceleration. In some experiments, erythrocyte pellets were formed in one medium and then the supernatant was gently replaced with another of a different ionic strength or osmolarity. Pronase-treated erythrocytes and pellets were also used for comparison purposes. All procedures were performed at room temperature unless otherwise noted.

Electron microscopy

Conventional transmission electron microscopy of erythrocyte pellets was carried out. Pellets formed on a plastic supporting film on the bottom electrode were fixed in 0.5 ml of 1.5% glutaraldehyde and then post-fixed with 1% OsO_4 in 0.1 M phosphate buffer. The fixed pellets on supporting film were removed from the chamber, dehydrated, and embedded in Epon-Araldite mixture. Ultrathin pellet sections were cut perpendicular to the supporting film, stained with lead citrate, and examined in a Hitachi H-600 electron microscope.

Electrical measurements and preliminary data analysis

Electrical measurements to determine the pellet resistance R_p and capacitance C_p were performed either during ($G > 1 \times g$) or after ($G = 1 \times g$) centrifugation. To avoid electrochemical polarization of the electrodes, periodic bipolar rectangular pulses U_o of amplitude ~500 mV and duration T of 10–50 μs were used.

When a rectangular pulses U_o was applied to the chamber, the actual potential U_{ch} across the chamber filled with a pure buffer or a cell suspension rose exponentially. This effect is caused by electrode polarization, which can be accounted for if an effective capacitance C_{el} of the order of 20 μF/cm² is assigned to the electrodes. Formation of a cell pellet changes the U_{ch} signal: it becomes more rectangular and has a relatively lower amplitude, indicating a simultaneous increase of the total chamber resistance ($R_p + R_{ch}$) and a decrease of the system capacitance $(1/C_p + 1/C_{el})^{-1}$, which approximately equals to the pellet capacitance C_p because $C_p \ll C_{el}$. Now the dependence U_{ch} on time t can be linearized in a form

$$f = \ln\left(\frac{R_p \cdot U_o - b \cdot U_{ch}}{R_p \cdot U_o + b \cdot U_T}\right) = -\frac{t \cdot b}{C_p \cdot R_p \cdot (R_l + R_{ch})}, \quad (1)$$

where U_T is U_{ch} at the pulse end and $b = (R_p + R_l + R_{ch})$.

Fig. 2 shows a typical system response U_{ch} to a low-voltage rectangular pulse U_o (500 mV, 50 μs) in its original and linearized (according to Eq. 1) forms. The straight line plotted in Fig. 2 *B* using linear regression fits ~80% of the original U_{ch} signal (*points*), with the variation coefficient 0.97. Thus,

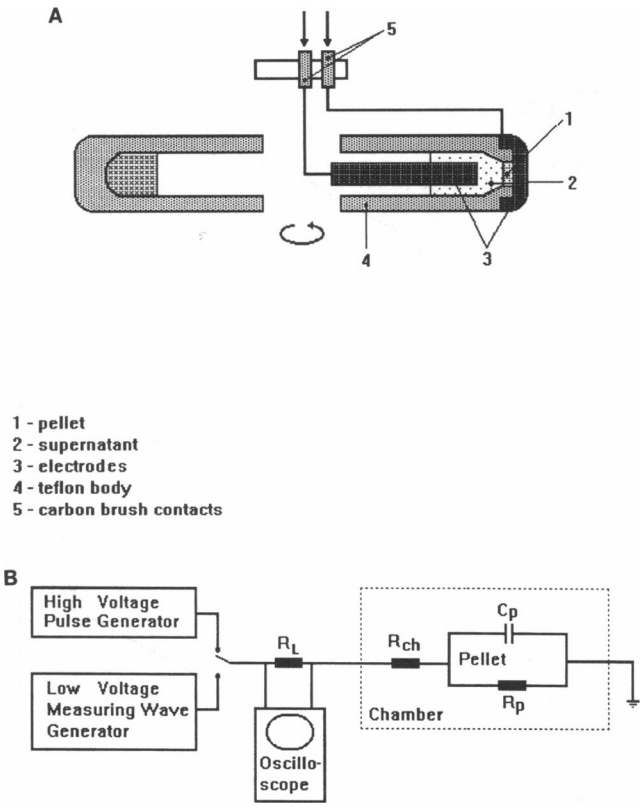

FIGURE 1 The experimental setup. (*A*) The centrifuge system showing only the chamber, counterbalance, and electrical contacts. The chamber on the right-hand side consists of two electrodes [*3*] attached to a Teflon body [*4*]. The cell suspension is centrifuged to form a pellet [*1*] on the lower electrode, leaving the supernatant [*2*] in contact with the upper electrode. Both electrodes are connected to respective carbon brushes [*5*] on the axle of the rotor. (*B*) The electrical circuit and the equivalent circuit of the chamber (within the dotted box). Details are given in the text.

Eq. 1 can be used to determine unknown parameters C_p and R_p of the pellet system. For sufficiently long pulses, R_p can be estimated even simpler as

$$R_p = U_{ch}/(U_{ch} - U_o) \cdot R_1 - R_{ch}. \quad (2)$$

Reproducibility

All qualitative regularities were well reproducible. However, measured values can vary with cell state. Therefore, unless otherwise noted, every figure includes data obtained with same cell samples, and all experiments were reproduced at least 2 times.

RESULTS

Dynamic response of erythrocyte pellets to external environment

Electrical measurements on erythrocyte pellets usually give reproducible results. However, sometimes transient and relaxation processes in cell pellets are so complicated and slow that cautions are necessary to be sure the system under study has really come to equilibrium. It should also be mentioned that from two measurable electrical parameters, the pellet resistance (R_p) and capacitance (C_p), the former is much more sensitive to experimental conditions, reflecting even minor changes in pellets. That is why this and the next papers are based mostly on R_p data.

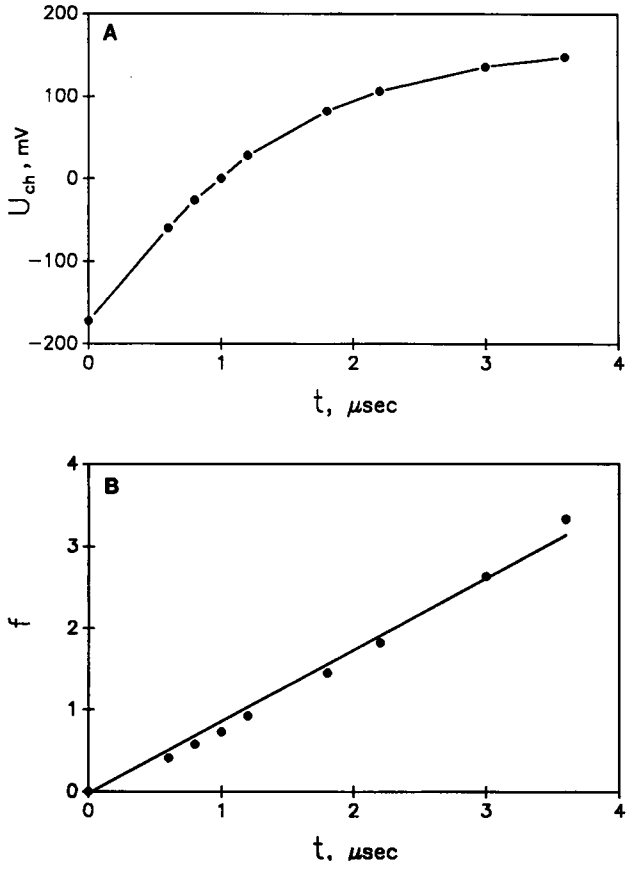

FIGURE 2 (*A*) A typical system response (U_{ch}) to a low-voltage rectangular pulse (U_o, 500 mV, 50 μs). (*B*) The same data plotted according to Eq. 1.

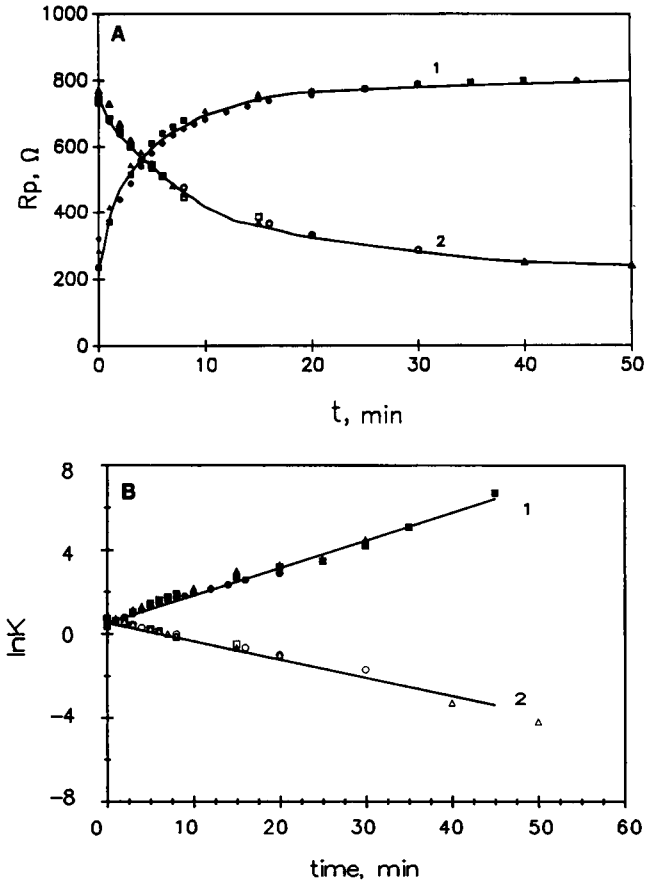

FIGURE 3 (*A*) Changes of the resistance of the pellet formed from 2×10^7 erythrocytes in isotonic PBS during ($900 \times g$, *curve* 1) and after ($1 \times g$, *curve* 2) centrifugation. Data from three centrifugation cycles are superimposed to show reproducibility: (●) first cycle; (▲) second cycle; (■) third cycle. (*B*) Data from Fig. 3 *A* plotted in semi-logarithmic coordinates.

Behavior of the pellet resistance under several routine manipulations with pellet systems is shown in Figs. 3–6. In Fig. 3 *A*, 2×10^7 erythrocytes suspended in isotonic PBS were pelleted at $G = 900 \times g$ for 20 min (*curve* 1), then centrifugation was terminated and the pellet was relaxed for 30–50 min (*curve* 2). This centrifugation-relaxation cycle was repeated 2 times more. As seen from the figure, the pellet resistance is reversible and reproducible through 3 cycles. Both $R_p(t)$ curves obey exponential kinetics because they are linear in semi-logarithmic coordinates (*curves* 1 and 2, Fig. 3 *B*). It should also be mentioned that, for thicker pellets, compression and relaxation are notably slower.

In Fig. 4, pellets formed from 2×10^7 erythrocyte were also subjected to three cycles of centrifugation with increasing G (*curves* 1–6); then the supernatant (isotonic sucrose-PBS contained 50 mM NaCl, 5 mM NaPi, 200 mM sucrose, and 300 mOsm) was replaced with either isotonic PBS (curves 7 and 8) or hypertonic (1400 mOsm) sucrose: PBS contained 40% PEG 8000 (*curve* 9). During the first centrifugation cycle ($G = 250 \times g$), R_p increased from 0 to ~1.6 kOhm and dropped to 0.75 kOhm after stopping the centrifuge. With increase of G to $500 \times g$ and then $900 \times g$ in the second and third cycles, R_p was nearly doubled, but returned

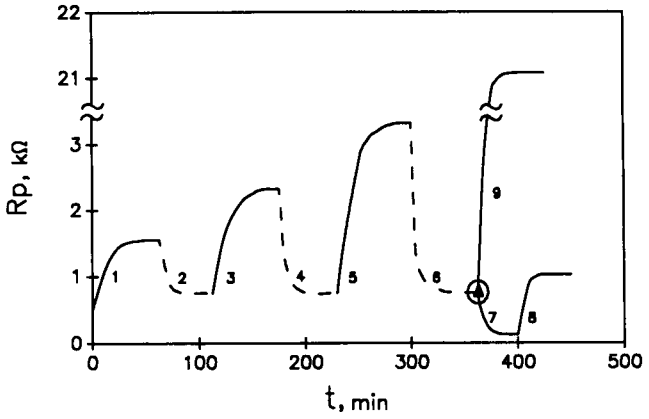

FIGURE 4 R_p of a 2×10^7 erythrocyte pellets under cycling centrifugation with increasing G (*curves* 1–6) in isotonic (300 mOsm) PBS-sucrose media (50 mM NaCl, 5 mM NaPi, 200 mM sucrose). Curves 1, 3, and 5 represent centrifugation of $250 \times g$, $500 \times g$, and $900 \times g$, respectively. Curves 2, 4, and 6 are relaxation at $1 \times g$. Changes of R_p are shown after replacing the supernatant with isotonic PBS (150 mM NaCl, 5 mM NaPi) (*curve* 7) and recentrifuge at $900 \times g$ (*curve* 8), and replacing the supernatant with hypertonic (1400 mOsm) PEG-containing solution (50 mM NaCl, 5 mM NaPi, 200 mM sucrose, 40% PEG 8 kDa) (*curve* 9).

to the same minimal level of 0.75 kOhm after the centrifuge stopped. The minimal values of R_p measured after centrifugation are called the resting pellet resistance, although no special notation is used. Most of the R_p data quoted in this paper, as well as values calculated from them, are referred to as the resting conditions unless otherwise indicated that measurements were made under centrifugation.

The last part of Figs. 4 and 5 show complicated response of erythrocyte pellets to changes in supernatant composition. The most prominent effect is a drastic increase (sometimes more than one order of magnitude) of the pellet resistance when a considerable amount of macromolecules like PEG is added to the supernatant (*curve* 9 in Fig. 4; curves 3 and 3′ in Fig. 5). Changes in concentrations of low-molecular weight particles, ions particularly, in supernatant can also significantly effect the pellet resistance. Thus, when isotonic sucrose-PBS (50 mM NaCl, 5 mM NaPi, 200 mM sucrose) is replaced with isotonic PBS (150 mM NaCl, 5 mM NaPi), R_p drops monotonically to a lower level, and upon further centrifugation, it increases to a level much lower than before supernatant replacement (compare *curves* 1, 2 and 7, 8 in Fig. 4). The same effect was observed when pellets were formed in isotonic PBS and then the supernatant was replaced with hypertonic (e.g., 900 mOsm) PBS (data are not shown). Generally, when the supernatant is replaced with a solution of higher electrolyte concentration, R_p drops monotonically down to a level as if a pellet were formed in this solution.

A different picture is observed, however, when the supernatant is replaced with a solution of lower electrolyte concentration. As seen from Fig. 5 (*curves* 4, 4′), in this case R_p first increases drastically and then gradually decreases to a very low level, much lower than that expected if pellets were formed directly from the hypotonic solution. The latter condition was confirmed by curves 5 and 6 obtained after resuspension and repelleting erythrocytes in hypotonic PBS.

Fig. 5 shows also that the pellet resistance increases considerably when erythrocytes are first subjected to pronase treatment, although this does not qualitatively change pellet behavior in different experimental conditions.

Another example showing the effect of the cell state on R_p is given in Fig. 6. Here a pellet was subjected to a single high-voltage pulse powerful enough to cause electroporation of cell membranes. Immediately after the pulse, the pellet resistance drops because cell membranes become, at least temporally, electroconductive (Kinosita and Tsong, 1977). However, R_p then rises to a very high level, exceeding the initial one several times. This drastic increase of the pellet resistance after pulse electrotreatment could be something

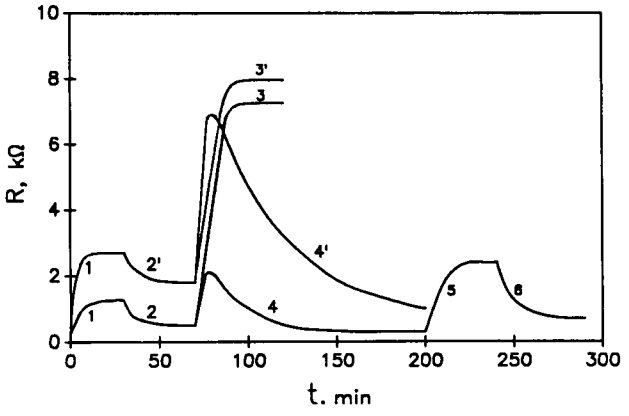

FIGURE 5 Effect of different manipulations with pellet systems on the resistance of pellets formed from 2×10^7 intact (*lower* curves) or pronase-treated (*upper* curves) erythrocytes in isotonic PBS. Erythrocytes were pelleted at $900 \times g$ for 30 min (*curves* 1 and 1′), then pellets were brought to equilibrium at $1 \times g$ (*curves* 2, 2′), the supernatants were replaced with either hypertonic (1400 mOsm) PEG-containing solution (8 kDa, 40% PEG added to isotonic PBS) (*curves* 3 and 3′), or hypotonic (200 mOsm) PBS (*curves* 4 and 4′). In addition, pellets from intact cells finally resuspended by shaking in hypotonic PBS and subjecting to a second centrifugation cycle (*curves* 5 and 6).

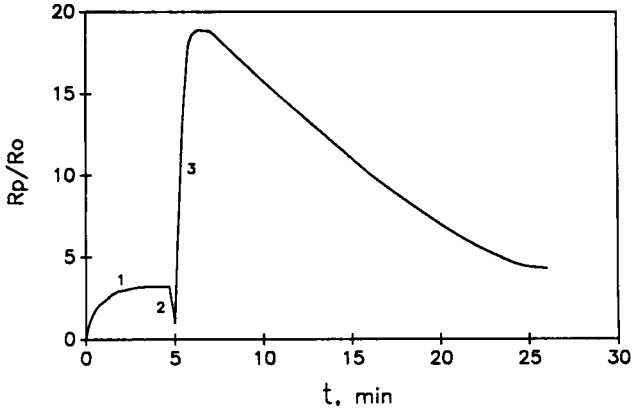

FIGURE 6 Effect of a single high-voltage pulse (250 V, 10 μs) on the resistance of the pellet formed from 2×10^7 erythrocytes in isotonic PBS.

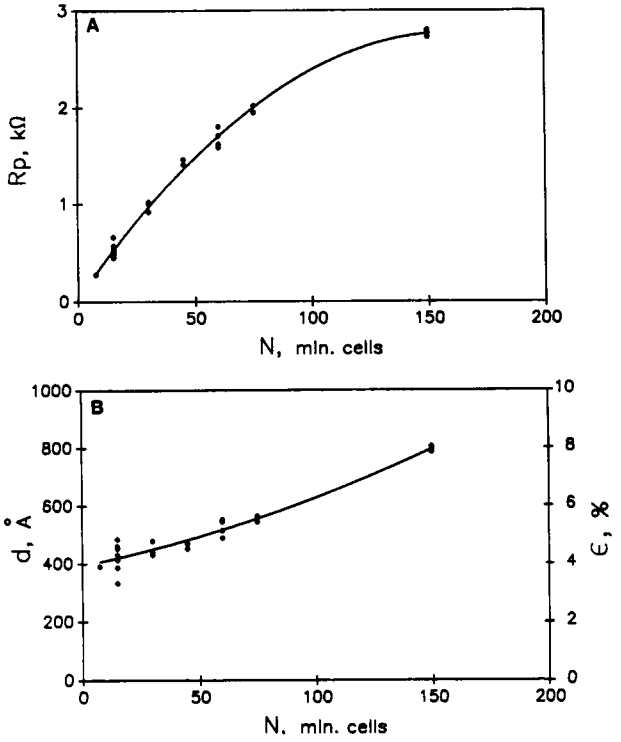

FIGURE 7 Dependence of the resting pellet resistance (*A*) and porosity and intermembrane distance (*B*) on the number of erythrocytes in pellets formed in isotonic PBS. (mln = million). The intermembrane distance *d* is derived from the porosity by using Eq. A.7.

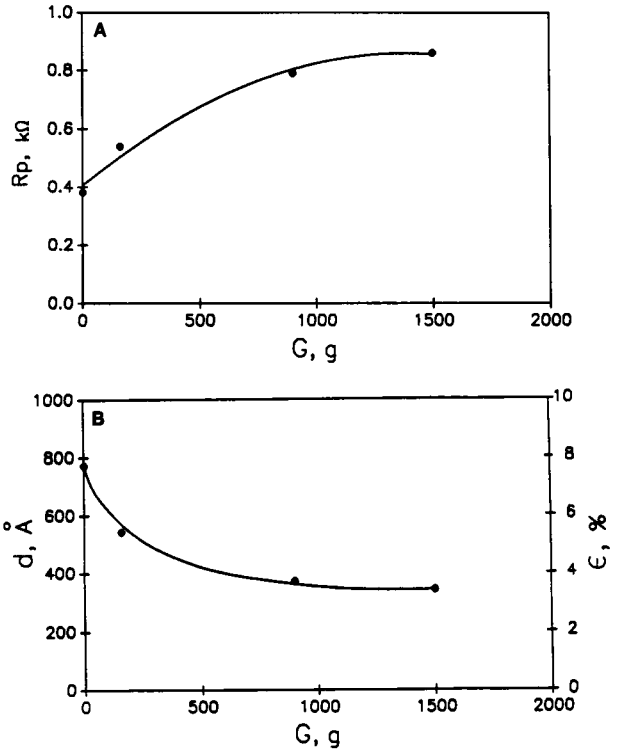

FIGURE 8 Effect of acceleration on the pellet resistance (*A*) and porosity and intermembrane distance (*B*) for 2×10^7 erythrocytes in isotonic PBS. The intermembrane distance *d* is derived from the porosity by using Eq. A.7.

unexpected if not for a similar effect observed earlier (Abidor et al., 1993) with pellets formed from different cells.

Electrical properties of erythrocyte pellets in equilibrium

In equilibrium, properties of erythrocyte pellets depend mainly on three factors: (i) the number of cells N in the pellet or the pellet thickness h; (ii) the acceleration G; and (iii) media composition.

Figs. 7 *A* and 8 *A* show that the pellet resistance R_p increases with N as well as G, although both dependencies are not linear as would be expected. The porosity ϵ and intermembrane distance d, deduced from R_p measurement as described in Discussion, are shown in Figs. 7 *B* and 8 *B*.

The number of cells is the only major factor affecting the pellet capacitance, as seen from Fig. 9, although the effect is very limited. An increase of N from 1 to 150×10^6 cells leads to a decrease of C_p from 1.4 to 0.5 nF. Other factors, like the acceleration, media composition, or different manipulations with the pellet systems cause only minor changes in C_p.

The dependence of R_p, from which corresponding ϵ and d are deduced, on the ionic strength of either PBS or PBS-sucrose suspending solutions is shown in Fig. 10. As seen, the higher is the electrolyte concentration, i.e., the higher solution electroconductivity, the lower is R_p.

Electron microscopy

Two typical electron micrographs of an erythrocyte pellet formed in isotonic PBS are given in Fig. 11. As seen from both micrographs, adjacent cell membranes form extended tight contacts with the intermembrane distance of ~100–300 Å. Only rarely is a space observed in cell junctions (Fig. 11 *B*). One should be cautious about these values because, as found in this study, adding glutaraldehyde in supernatant leads to a notable increase of the pellet resistance. Although this increase is attributable to the impediment of current flow through the intercellular space, caused by protein cross-linking by the fixative, it might also involve structural change. It should be noted that the free space in cell junctions is very small or absent altogether. These electron micrographs confirm that erythrocyte pellets are packed very tightly as analyzed below on the basis of electrical measurements.

DISCUSSION

Results of this and previous (Abidor et al., 1993) studies show that electrical properties of cell pellets, the pellet resistance in particular, are sensitive to experimental conditions affecting the pellet structure. This paper describes how the electrical properties are related to structural characteristics of erythrocyte pellets, and how to derive structural parameters of the pellet from electrical measurements. Osmotic effects, electroporation, cell membrane interactions, and other related phenomena observed in cell pellets are considered in the following paper.

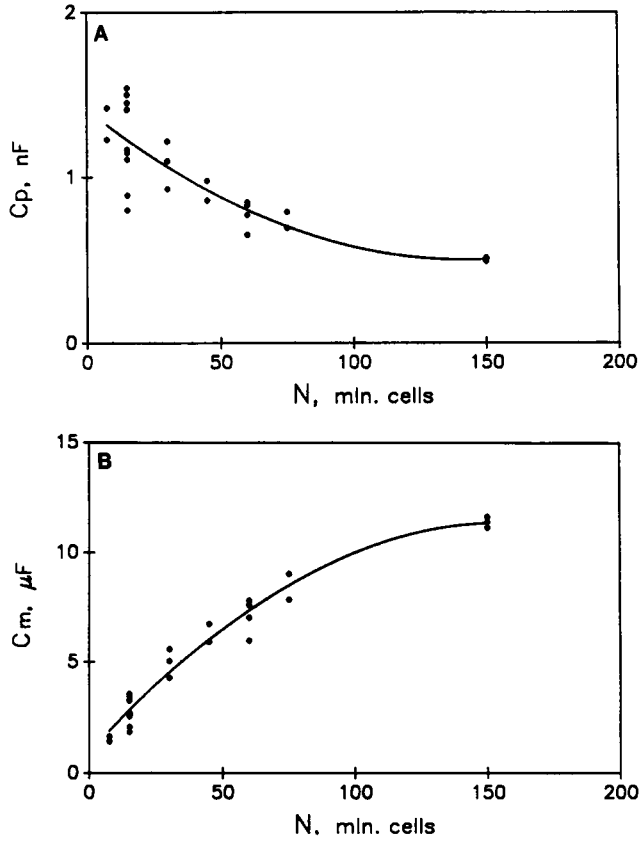

FIGURE 9 Dependence of the pellet capacitance (*A*) and cell membrane capacitance (*B*) on the number of erythrocytes in pellets formed in isotonic PBS.

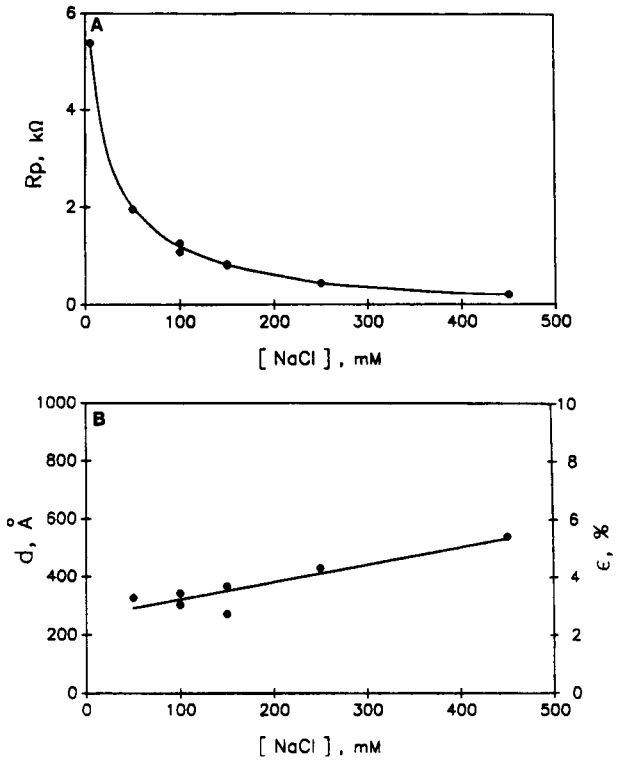

FIGURE 10 Effect of NaCl concentration in PBS or PBS-sucrose on the pellet resistance (*A*) and porosity and intermembrane distance (*B*) for 2×10^7 erythrocyte pellets. The intermembrane distance d is derived from the porosity by using Eq. A.7.

To classify erythrocyte pellets as a physicochemical system, one has to consider that erythrocytes are easily deformable, and can be expected to be packed in compact pellets. Electron micrographs given in Fig. 11 confirm that there are no significant intercellular cavities in cell junctions. Therefore, pellets can be treated neither as conventional porous bodies, in which positions of the materials are fixed, nor as granular materials that consist of free, easily movable particles (Jaeger and Nagel, 1992). Moreover, intercellular space might be impermeable to macromolecules because of overlapping glycocalyx. In this sense, erythrocyte pellets are more similar to gels (McBain, 1950) than to either granular or conventional porous materials.

Reproducibility and reversibility of the pellet resistance show that erythrocyte pellets can be brought into thermodynamic equilibrium. It is worth noting, however, that transient and relaxation processes in cell pellets can be quite slow. As seen from Fig. 3 *B*, during and after centrifugation, R_p changes exponentially in time with characteristic times τ of the order of several minutes. τ for erythrocyte pellets depends on the compressing force because it increases with the pellet thickness and decreases with the centrifuge acceleration (data are not shown). Therefore, the transient and relaxation processes in erythrocyte pellets are determined also by material exchange between pellets and supernatant rather than by pellet elastic properties alone, as depicted by the Voigt's body model.

After considering briefly the rheological behavior of erythrocyte pellets, we can now discuss the relationship between electrical properties and structures of pellets. As seen from Fig. 2 *B*, erythrocyte pellets can be represented by a simple equivalent circuit consisting of a capacitance, C_p, and a resistor, R_p, in parallel. It is natural to assume, as in the Appendix, that C_p depends mainly on the specific capacitance C_m and relative positions of cell membranes, whereas R_p is determined by intercellular conductivity and porous structure. However, are these assumptions valid?

In terms of the simplest model describing cell pellets as a system consisting of L layers of flat-parallel cells, C_p should be proportional to C_m/L (Eq. A.10). At a first glance, the function $C_p(L)$ in Fig. 9 *A* is indeed hyperbolic as expected from this relation. However, a more careful analysis shows that this function is not exactly hyperbolic, because calculated values of C_m rise significantly with L, as seen from Fig. 9 *B*. It is noteworthy that, for small numbers of cell layers, the calculated value of C_m ($\sim$1.3 μF/cm^2) is quite close to the expected C_m for erythrocytes (0.8 to 0.9 μF/cm^2), whereas for thicker pellets, calculated C_m becomes several times higher. Two possible explanations of this effect are proposed. First, there is some additional (although unknown) polarization effect in cell pellets, and second, cells may change orientation with the pellet thickness (in the latter case, cells are not considered to be cubic).

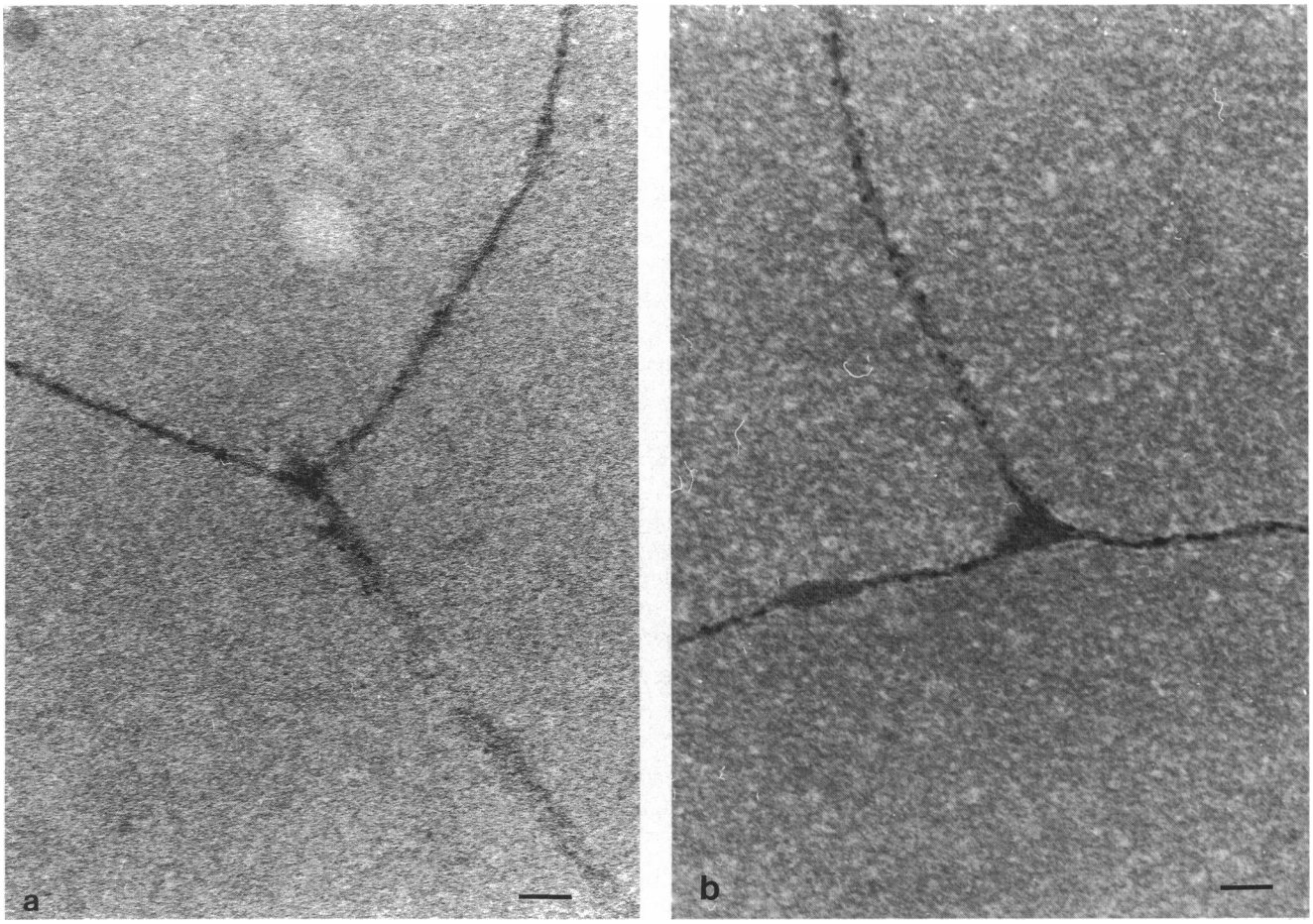

FIGURE 11 Electron micrographs of a cell pellet formed by 2×10^7 rabbit erythrocytes at $900 \times g$, showing tight cell contacts (*a*) and rare occasional space in cell junctions (*b*). Bar = 0.1 μm.

Possible changes in cell orientation or packing with the pellet thickness are also reflected in the dependence of R_p on N given in Fig. 7 *A*. A notable decrease of the slope with N merely shows that erythrocyte pellets become looser as pellets get thicker. This effect is clearly not related to the cell membrane conductivity. Indeed, even for multilayer pellets, R_p does not exceed several kOhms, which is many orders lower than the resistance expected from a pellet consisting of one continuous cell membrane layer (the specific membrane resistance is $\sim 10^8$–10^9 Ohm/cm^2 (Schwan, 1957)). Therefore, R_p must depend mainly on the conductivity of intercellular space filled with aqueous solution, rather than on the cell membrane conductivity. The question is: how is R_p related to the intercellular conductivity and structural characteristics of cell pellets?

Unfortunately, despite the vast literature on electrical properties of porous bodies, there is still no general theoretical approach to this problem. In fact, quantitative analysis of porous bodies is possible only in some particular cases when the porous structure is simple and regular. Fortunately, erythrocyte pellets composed of cells of definite volume v and surface area s can be modeled as a simple, regular porous structure.

To describe porous structures, two major parameters are usually used: the characteristic pore size d (for common porous bodies, d is usually the average pore radius; for cell pellets with extended narrow gaps between contacting cell membranes, d is the average intermembrane distance) and the porosity ϵ (defined as an intercellular volume/total pellet volume ratio, V_p/V). As shown in the Appendix, d is related to v, s, and ϵ by Eq. A.7, which can be rewritten for practical use in the form

$$d = 200 \cdot \epsilon \cdot (v/s), \tag{3}$$

where d is given in Å, ϵ is given in %, v is given in μm^3, and s is given in μm^2. As follows from Eqs. A.5 and A.15, the pellet porosity ϵ can be related to several electrical and geometric parameters (the pellet resistance R_p, the intercellular conductivity λ, the pellet thickness h, and base area S) as follows:

$$\lambda = k \cdot h/\epsilon \cdot R_p \cdot S, \tag{4}$$

where k is a coefficient of values between 1.5 and 2.25. In the first approximation, it is reasonable to accept $k = 1.75$ (as found in the Appendix for model pellets consisting of cubic cells packed in layers randomly positioned relative to one another). All other values on the right side of Eq. 4 are basically known: S equals the working area of the chamber

hole; h can be calculated (because the number of cells in the pellet N is usually known) by means of Eq. A.5, although in principle it can also be measured directly; R_p is measured quite precisely; and λ should be close to the conductivity of the bulk solution (effects of the surface conductivity and steric obstacles from the glycocalyx on λ hardly exceed 20–30%, as shown in the following paper).

Calculated ϵ and d for different equilibrium conditions are given in Figs. 7, 8, and 10. The values of ϵ varying in the range of 1–10%, and d in the range of 100–800 Å, can be regarded as upper limits. Thus, all of our data indicate that pellets formed from rabbit erythrocytes are quite compact. As seen from Figs. 3–5, the pellet porosity and intermembrane distance can decrease several times with the centrifuge acceleration. Both parameters increase slowly with the pellet thickness (Fig. 7 *B*) (as can be expected from $R_p(N)$ dependence discussed above), showing once more that the thicker the pellets the looser they are. However, the most dramatic changes in the pellet structure are caused by variations of the supernatant osmolarity or ionic strength (Figs. 5 and 10) or by the application of a high-voltage electric pulse to the pellet (Fig. 6). Thus, after adding 40% PEG to the supernatant, ϵ and d dropped to 0.2% and 23 Å, respectively. A similar result was observed after the application of one pulse of 250 V, 10 μs to a pellet formed from 2×10^7 erythrocytes in isotonic PBS. These predominantly osmotic effects will be discussed in detail in the next paper, whereas here they are shown only to demonstrate the variability of the pellet structure.

The above calculations are rather rough and suffer some uncertainties. For instance, (i) the division of cell pellets into separate cell layers is not entirely justified; (ii) the choice of the geometric factor k to be 1.75 is not exact; and (iii) the solution conductivity between cells is probably higher than that of the bulk solution (the surface conductivity effect is hardly significant unless $d < 100$ Å). Nevertheless, this approach gives at least some insight into the cell pellet structure and helps to understand better their behavior under different conditions, as shown in the next paper.

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APPENDIX

Main structural characteristics of erythrocyte pellets

Rabbit erythrocytes suspended in isotonic solutions are normally discocytes with an average diameter $d_c = 7.3$ μm, the greatest thickness $R_c = 1.7$ μm, volume $v_o = 57$ μm^3, and surface $s = 110$ μm^2 (Ponder, 1971). The cell surface s is constant, whereas all other geometric parameters are highly dependable on experimental conditions. In particular, v depends on the media osmotic pressure P as (Sachs et al., 1975):

$$v = v_o \cdot (\omega_h + (1 - \omega_h) \cdot P_o/P), \tag{A1}$$

where subscript o refers to isotonic conditions and ω_h (~0.4) is a fraction of the cell volume occupied by hemoglobin molecules in isotonic conditions.

Now let us consider an erythrocyte pellet formed from N cells. Because of the shape of the chamber, the pellet is a thin disk with the base area S and the thickness h. To describe the structure of porous bodies, two geometrical characteristics, the total pore surface area S_p and the porosity ϵ, are commonly used. For cell pellets,

$$S_p = N \cdot s, \tag{A2}$$

whereas ϵ is a dimensionless value defined as a ratio of the intercellular space volume V_p to the total pellet volume V.

$$\epsilon = V_p/V \tag{A3}$$

Usually $V_p \ll V$, hence,

$$V = N \cdot v, \tag{A4}$$

and

$$h = V/S = N \cdot v/S. \tag{A5}$$

Because in erythrocyte pellets pores look like extended two-dimensional gaps, the average intermembrane distance d equals

$$d = 2 \cdot V_p/S_p. \tag{A6}$$

Combining Eqs. A.2–A.4 and A.6 we obtain

$$d = 2 \cdot \epsilon \cdot (v/s). \tag{A7}$$

The last equation relates d with several experimentally defined parameters: the cell volume v and surface area s are basically known (see above), and the pellet porosity ϵ can be estimated from the pellet electrical resistance as shown below.

Electrical properties and the structure of erythrocyte pellets

Although electrical properties of porous bodies soaked in electrolyte solutions have been studied intensively, their theoretical analysis is still incomplete. Generally, quantitative description of any new system requires modeling with serious simplifying assumptions on porous structures (Johnson et al., 1986). Thus, a suitable model of erythrocyte pellets should be developed first.

As follows from our results, erythrocyte pellets are packed very tightly, without free space in cell junctions. To be so compact, cells must lose their regular discocyte shape. Electrical characteristics of erythrocyte pellets, particularly the resistance R_p and the capacitance C_p, depend on the number of cell layers L in the pellet which, in turn, depends on cell shape and orientation. Although cell layers in pellets are not well defined and, moreover, are intercalated, to the first approximation one might suppose that, *on average*, pelleted cells look like small cubes packed regularly in layers parallel to the bottom electrode. In isotonic conditions, erythrocytes can be easily deformed into regular cubes because their characteristic size as calculated either from the cell volume ($a = v^{1/3} = 3.85$ μm) or from the cell surface ($a = (s/6)^{1/2} = 4.15$ μm) are quite close ($a \approx 4$ μm). In this case, the number of cell layers L equals

$$L = \frac{N \cdot a^2}{S}. \tag{A8}$$

Knowledge of L is important when electrical properties of cell membranes are related to similar characteristics of cell pellets. Thus, when a voltage U_p is applied to a pellet system, the maximal voltage drop across cell membranes normal to the electric field equals

$$\omega_m = \frac{U_p}{2 \cdot L}. \tag{A9}$$

Similarly, the specific membrane capacitance can be estimated from the

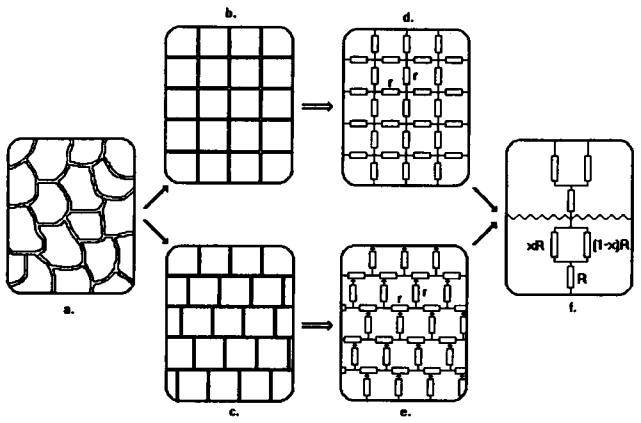

FIGURE 12 Transition from a real pellet structure (*a*) to a generalized equivalent scheme (*f*) of erythrocyte pellets. Pictures *b* and *c*, and *d* and *e* show structures and equivalent circuits of cubic cells packed in regular and irregular (shifted) arrays, respectively. $R = r/n$ is the resistance of a cell layer consisting of n cells; r is a resistance of a single cell contact; x is a relative shift between neighbor layers ($0 \le x \le 1$).

pellet capacitance C_p as

$$C_m = \frac{2 \cdot C_p \cdot L}{S}. \tag{A10}$$

To describe quantitatively the pellet resistance R_p, let us first assume that cells form a regular array, shown in Fig. 12 *a*. The pellet resistance R_p depends on the "pore" conductivity λ, the pellet thickness h, and the total area of "pores" A_p in the plane normal to the electric field, i.e.,

$$R_p = \frac{h}{\lambda \cdot A_p}. \tag{A11}$$

For the discussed model, the product $h \cdot A_p$ equals $\frac{2}{3} \cdot V_p$. Inserting this condition together with Eq. A.3 in Eq. A.11 and taking into account that $V = S \cdot h$ we have

$$R_p = \frac{\beta \cdot h}{\lambda \cdot S \cdot \epsilon}, \tag{A12}$$

where $\beta = 1.5$. Thus, in this regular model only two-thirds of the total pore volume takes part in pellet conductivity, which agrees with an equivalent scheme shown in Fig. 12.

A more realistic model is a pellet consisting of the same cell layers with some shift in respect to each other. In this case, the pellet resistance is increased because electrical connection between neighbor layers now occurs through gaps normal to the main direction of the electric field. This effect can be taken into account by adding two parallel resistances, $R \cdot x_i$ and $R \cdot (x_i - 1)$, formed by proportional division of the total gap resistance to the resistance of each layer, R, in series as shown in Fig. 12. Here x_i is a dimensionless parameter laying between 0 and 1 and showing the average relative shift of the ith layer in respect to the $(i + 1)$th layer. It is easy to show that, for an averaged relative interlayer shift x, the pellet resistance R_p should be multiplied by a factor

$$\alpha(x) = 1 + \frac{L - 1}{L} \cdot x \cdot (x - 1)$$

or, because usually $L \gg 1$,

$$\alpha(x) = 1 + x - x^2. \tag{A13}$$

As could be expected, the function $\alpha(x)$ equals 1 for $x = 0$ or 1, i.e., for regular cubic lattice, and reaches a maximum (1.5) at $x = 0.5$, i.e., when each cell layer is shifted with respect to neighbor layers at half the elementary cubic cell. It is reasonable to expect that in a multilayer pellet, values of x are evenly distributed between 0 and 1; thus, the mean value of $\alpha(x)$ equals

$$\alpha = \int \alpha(x)\,dx \Big/ \int dx = 1.166. \tag{A14}$$

For arbitrarily packed multilayer pellets formed of cubic cells,

$$R_p = \frac{k \cdot h}{\lambda \cdot S \cdot \epsilon}, \tag{A15}$$

where $k = \alpha \cdot \beta = 1.166 \cdot 1.5 \approx 1.75$.

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