ANOMALOUS DIFFUSION OF ERYTHROCYTES IN THE PRESENCE OF POLYVINYLPYRROLIDONE

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ABSTRACT The diffusion coefficient of erythrocytes was measured using quasi-elastic light-scattering (QELS) techniques. The cells were suspended in phosphate-buffered saline solutions with and without a macromolecule, polyvinylpyrrolidone (PVP[360]). In the presence of the PVP(360) an anomalously high diffusion coefficient was measured for metabolizing cells with a normal transmembrane potential. The results are in agreement with experiments on rouleau formation by red blood cells and are supportive of the hypothesis of a long-range coherent interaction between metabolically active biological cells.

INTRODUCTION

Over the past several years a hypothesis concerning the possible coherent interaction of dipoles located in cellular membranes has been developed. The primary impetus for this was a paper by H. Fröhlich (1). An extensive review of the subject is also given by H. Fröhlich (2). According to the hypothesis, the thermal vibrations of giant dipoles in the cell membrane should exhibit a "Bose-Einstein condensation" when they experience an energy input above a critical rate. This input can either be from an external source or from the cellular metabolism itself. As a result of this Bose-Einstein condensation, a long-range intercellular interaction with a resonance frequency of the order of 10^{11} Hz is conjectured. Therefore much of the search for experimental evidence of this interaction has involved microwave or Raman spectroscopy (3–5). Other experimental techniques, which also require an external energy input, have been used (6, 7). However, with an external energy input, it is usually difficult to separate ordinary thermal effects from the hypothesized coherent interaction (2).

Recently Rowlands et al. (8–10) have produced experimental data that they interpret to support the existence of a Fröhlich-type of interaction between red blood cells (erythrocytes). The experimental technique used by Rowlands et al. (8–10) does not require an external energy input but relies on the metabolism of the erythrocyte. Their data are based on the tendency of erythrocytes to stick together when they come into contact under appropriate conditions. This process of sticking together is referred to as "rouleau formation."

The formation of rouleau is a diffusion-controlled reaction and can be treated as an example of the coagulation of Brownian particles. The general problem of coagulation of Brownian particles is considered theoretically by Smoluchowski (11) and is extended to include interacting particles by Fuchs (12). A general review of the theory is found in Chandrasekhar (13). Swift and Friedlander (14) investigate the coagulation of hydrosols experimentally for both the hard-sphere case, originally considered by Smoluchowski, and for the interacting particle case for which Fuch's extension of the theory is required.

Basically Rowlands et al. (8–10) found the rate of rouleau formation could not be explained by the Brownian motion of noninteracting particles. It was necessary to assume that the presence of a long-range attractive potential between the erythrocytes accounted for their data. They observe that this interaction potential disappeared if either the metabolism or the transmembrane field of the erythrocyte is abolished. These changes are reversible.

Their initial observations were made with the red blood cells suspended in plasma, but later it was found (15) that similar phenomena occurred in artificial media, such as a phosphate-buffered saline, if a suitable macromolecule was present above a critical concentration. (Presumably fibrinogen is the required macromolecule in plasma suspensions.)

Because the rate constant for rouleau formation is derived from a diffusion coefficient (13), a direct measurement of the diffusion coefficient of erythrocytes under the same physiological conditions would be of interest. Quasi-elastic light scattering (QELS) has become a standard technique for the measurement of diffusion coefficients for particles satisfying the Rayleigh-Gans-Debye criteria: $k|(m-1)|d \ll 1$, where k is the magnitude of the wave vector, m is the relative refractive index, and d is a characteristic dimension of the scatterer. There are many reviews in the literature on this technique. A recent review with references to earlier reviews is Bloomfield (16).

More recently the techniques of QELS have been applied to particles of micron size such as spermatazoa and *Escherichia coli* (17–19). A theoretical treatment that justifies the extension of light-scattering techniques to micron-size particles has been given (20).

EXPERIMENTAL METHODS

Light Scattering Apparatus and Methods

The principles and techniques of QELS are well documented (21, 22, 16). The apparatus used in this study has been described earlier (23). The 488-nm line of an Ar-ion laser (Coherent Inc., Palo Alto, CA) was focused onto a suspension of erythrocytes and the light scattered by the red blood cells at an angle of θ was detected through a pair of collimating pinholes by a photomultiplier (9558QB; EMI, Middlesex, England). The photocurrent was amplified by a preamplifier (PAR 113) and fed to a correlator (SAICOR 43A; Honeywell, Inc., Test Instruments Div., Denver, CO). The autocorrelation function of the photocurrent produced by the correlator was coupled directly to a computer (Apple II; Apple Computer Inc., Cupertino, CA) where the data analysis was performed.

The autocorrelation function of the photocurrent can be shown

$$C'(t) = A + B \exp(-2\Gamma t), \quad (1)$$

where Γ is the decay constant of the autocorrelation function and A and B are independent of time. The quantity symbolized by Γ is related to the diffusion coefficient, D, by

$$\Gamma = q^2 D = [(4\pi n/\lambda_o) \sin(\theta/2)]^2 D, \quad (2)$$

where the factor in the square brackets is the magnitude of the scattering vector. The scattering angle is θ, λ_o is the vacuum wavelength of the incident light, and n is the refractive index of the scattering media. The quantity dealt with in the remainder of this paper is

$$C(t) = C'(t) - A. \quad (3)$$

The constant A is obtained as the asymptotic value of $C'(t)$ as t becomes very large.

Sample Preparation

Blood was collected from the author into a heparinized evacuated glass container (Becton, Dickson and Co., Columbus, NE) by venipuncture. It was centrifuged immediately to separate the plasma from the erythrocytes. The plasma was then discarded and replaced by 0.15 M NaCl. The erythrocytes were gently stirred in the saline, recentrifuged, and again the supernatant was discarded. This procedure was repeated until the supernatant was clear. The cell suspension was stored at 2°C. Aliquots of the suspension were drawn off and added to the desired solutions, gently mixed, allowed to warm to room temperature, and then placed in the light-scattering apparatus. In most experiments the erythrocytes were used the same day they were collected, and samples more than four days old were discarded.

Solutions

The standard solution in which the cells were suspended for the light-scattering experiment was a phosphate-buffered saline solution to which human serum albumin (HSA) had been added. The composition of the solution, hereafter called PBS, was: 150 mM NaCl, 7.3 mM Na_2HPO_4, 2.8 mM NaH_2PO_4, 0.3 g/100 ml HSA. The pH of the PBS was 7.4.

As mentioned in the Introduction, the presence of a macromolecule is necessary to have rouleau formation in PBS. The macromolecule used in this work was polyvinylpyrrolidone (PVP) (Sigma Chemical Co., St. Louis, MO). Three different molecular weights of the PVP were used: 360,000, 40,000, and 10,000, hereafter referred to as PVP(360), PVP(40), and PVP(10), respectively. The PVP, as received, was added to portions of the PBS to a concentration of 0.3 g/100 ml.

When it was desired to lower the pH of the cell suspension, a solution of 0.15 M HCl plus 0.15 M NaCl was slowly added with constant stirring. The pH was restored to 7.4 by adding 0.15 M NaOH (25). Metabolically depleted erythrocytes were obtained by preparing cell suspensions in either PBS or PBS plus PVP and incubating them for 24 h at room temperature. Metabolic activity was restored by adding adenosine to the suspension and incubating the suspension for an additional 3 h at room temperature (26).

All solutions were filtered through a 0.2 μm Nalgene filter unit (Nalge Co., Rochester, NY) before they were used. They were all stored at 2°C and none were used that were more than three weeks old. The viscosities of all the solutions used were measured at room temperature in a Ostwald viscosimeter (Sargent-Welch Co., Skokie, IL). The viscosities relative to water are tabulated in Table I.

The cell suspensions were lightly colored and therefore there was a question of heating due to the incident light. A thermocouple was placed in a cell suspension a known distance from the incident beam. From the increase in the temperature of the thermocouple in the steady state and the geometry of the sample cell, it was estimated that the temperature in the beam was no more than 2°C above room temperature.

HSA itself is a macromolecule and PVP is known to form colloidal suspensions in aqueous solutions. Therefore the solutions were examined for light scattering without added erythrocytes. In all blank solutions, the scattered light intensity was at least two orders of magnitude lower than with the erythrocytes.

Potential Sources of Artifact

The large size of the human erythrocyte caused some initial concern for several reasons. (*a*) The Rayleigh-Gans-Debye criteria were not satisfied; (*b*) they tended to sediment producing a directed component to the velocity; (*c*) as a result of the sedimentation there would tend to be a time-dependent concentration in the vertical direction; and (*d*) because of their large size, multiple scattering would be observed unless the number concentration was kept very low. But then there was the possibility that number fluctuations within the scattering volume would make the measurements ambiguous. These concerns will be discussed in sequence.

(*a*) It was pointed out in the Introduction that QELS has been applied to micron-size particles. Indeed one of the larger particles to which QELS has been applied is a bull spermatazoan whose ellipsoidal head has semiaxes of 0.5, 2.3, and 4.5 μm (27). Even though these dimensions are comparable with the erythrocyte, it was desirable to calibrate the apparatus using 8-μm diam polystyrene DVB microspheres (Duke Scientific Corp., Palo Alto, CA). A suspension of spheres was prepared in a 1% sodium dodecylsulphate solution and the autocorrelation function of the scattered light intensity for this suspension was obtained. The quantity $C(t)$ was plotted as a function of q^2t on log-linear paper. It was observed that ln $C(t)$ was a linear function of q^2t and the diffusion coefficient of the 8-μm spheres was calculated to be 6.0×10^{-10}cm^2/s. This is in reasonable agreement with the Stokes-Einstein value of 5.4×10^{-10}cm^2/s.

(*b*) Studies of motile organisms using QELS have shown that a directed component superimposed on the random Brownian motion of the scattering particle causes ln $C(t)$ to be a nonlinear function of q^2t. However, it is the quantity $\mathbf{q} \cdot \mathbf{v}$, where $\mathbf{v}$ is the directed velocity that is effective in producing the departure from linearity. In these experiments the scattering vector is in the horizontal plane and, in the main, the directed velocity is expected to be in the vertical plane. Thus $\mathbf{q} \cdot \mathbf{v}$ should be nearly 0.

TABLE I
RELATIVE VISCOSITIES OF THE SOLUTIONS USED TO SUSPEND ERYTHROCYTES

Solution	Relative viscosity
PBS, pH 7.4	1.05
PBS, pH 6.3	1.03
PBS + PVP (360), pH 7.4	1.61
PBS + PVP (360), pH 6.3	1.56
PBS + PVP (360), pH 7.4(2)	1.51
PBS + PVP (40), pH 7.4	1.06
PBS + PVP (10), pH 7.4	1.03

It is conceivable, however, that as the particles settle there could be a helical or propeller screw-type motion that would give a nonzero value to $\mathbf{q} \cdot \mathbf{v}$. In this event ln $C(t)$ would not be a linear function of q^2t since it has been shown that such motion produces an autocorrelation function independent of the scattering angle and proportional to t^2 (28). Because ln $C(t)$ was observed to be linear in q^2t (see the data indicated by triangles in Fig. 3), within experimental error, it is felt that the directed motion of the erythrocytes caused by sedimentation did not contribute significantly to the overall autocorrelation function of the photocurrent.

(*c*) It is implicit in the treatment of QELS that the concentration of the scatterers in the scattering volume is constant. Since the erythrocytes tend to sediment from an aqueous solution in a gravitational field, it is clear that the concentration of cells will not be a constant in the vertical direction. However, the sedimentation velocity is of the order of 1 μm/s in PBS and the duration of any single scattering experiment is <10 min. Moreover, the scattering volume is located approximately in the center of a 2-cm column of sample. Therefore, no significant change in the concentration of scatterers in the scattering volume during a given experiment is expected.

(*d*) Multiple scattering becomes significant when the concentration is large enough so that the total scattered intensity is no longer a linear function of the concentration. For 0.312-μm spheres used by Colby et al. (29), this occurred at a concentration of $\sim 5 \times 10^{-5}$ in units of volume of spheres per unit volume of suspension. As the concentration increased and multiple scattering became more significant, the apparent diffusion coefficient increased. Fig. 6 of Colby et al. (29) shows that after the onset of multiple scattering the reciprocal of the apparent diffusion coefficient is a linearly decreasing function of the concentration.

Fig. 1 shows the scattered light intensity as a function of the concentration of red blood cells at a scattering angle of 60°. Clearly at a concentration of 5×10^{-4} vol/vol, there is a significant departure from linearity. Based on a measured hematocrit and an erythrocyte count, the mean volume of the red blood cells used in this work was 92.8×10^{-12} cm^3. Thus multiple scattering is already evident at a cell concentration of 5.4×10^6 cm^{-3}.

The scattering volume of the apparatus is of the order of 2.4×10^{-6} cm^3, so at the onset of multiple scattering there are only 12 or 14 cells in the scattering volume. Generally, number fluctuations do not become significant until the mean number of scatterers in the scattering volume is of the order of unity (30, 31). So to avoid the problems connected with multiple scattering and number fluctuations a cell concentration of $\sim 2 \times 10^6$ cm^{-3} was used. It varies somewhat because of the dilutions involved in changing the pH and adding adenosine to restore the metabolic activity.

Fig. 2 is a semilog plot of $C(t)$ vs. q^2t for various concentrations of 8-μm spheres in aqueous suspension. The nonexponential behavior of the correlation function at the highest concentration is evident. Presumably this is due to multiple scattering. At the lowest concentration the beginnings of a slowly decaying tail can be seen at large values of q^2t. This is ascribed to number fluctuations. At the intermediate concentration a reasonably good exponential decay is observed for $C(t)$. Fig. 3 is a semilog plot for erythrocytes suspended in PBS + PVP(10). All the same characteristics are observed as in Fig. 2.

Because number fluctuations contribute to the autocorrelation function independent of the scattering angle (28) and because multiple scattering is angle dependent (29), it is unlikely the linearity in Figs. 2 and 3 is produced by compensating effects. It may be concluded that it is feasible to use QELS to monitor the diffusion coefficient of particles as large as 8-μm diam spheres. In particular, the technique seems applicable to erythrocytes if the concentration of cells is chosen to avoid both multiple scattering and number fluctuations.

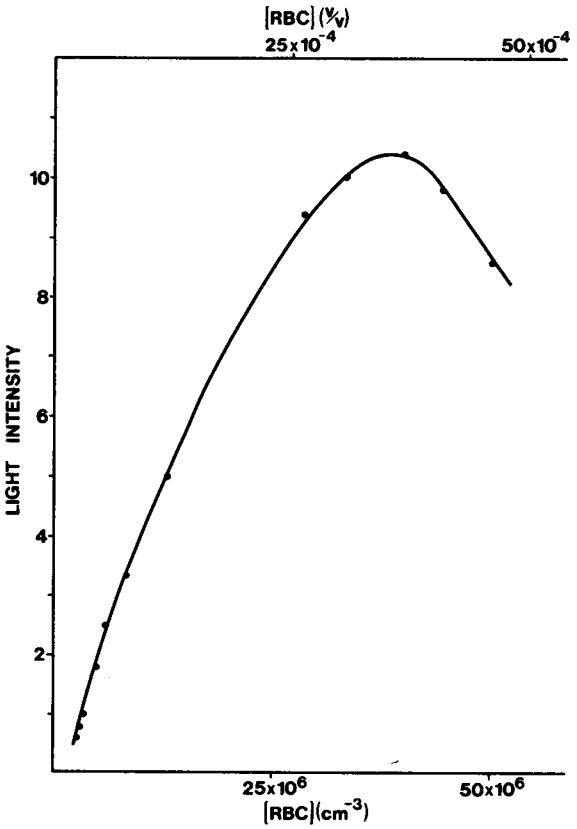

FIGURE 1 Scattered light intensity as a function of concentration of human red blood cells in PBS at 60°. The vertical axis is in arbitrary units.

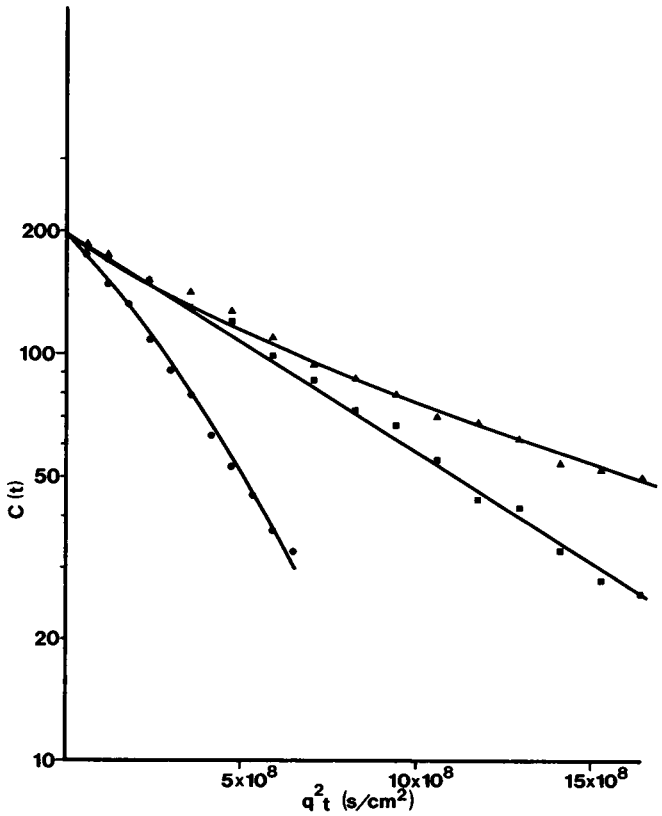

FIGURE 2 A semilog plot of $C(t)$ vs. q^2t for 8 μm polystyrene DVB microspheres at various concentrations. The scattering angle is 90°. The triangles (▲) indicate a concentration of spheres of 0.62×10^6 cm^{-3}, the squares (■) are for a concentration of 0.83×10^6 cm^{-3}, and the circles (●) represent a concentration of 1.34×10^6 cm^{-3}.

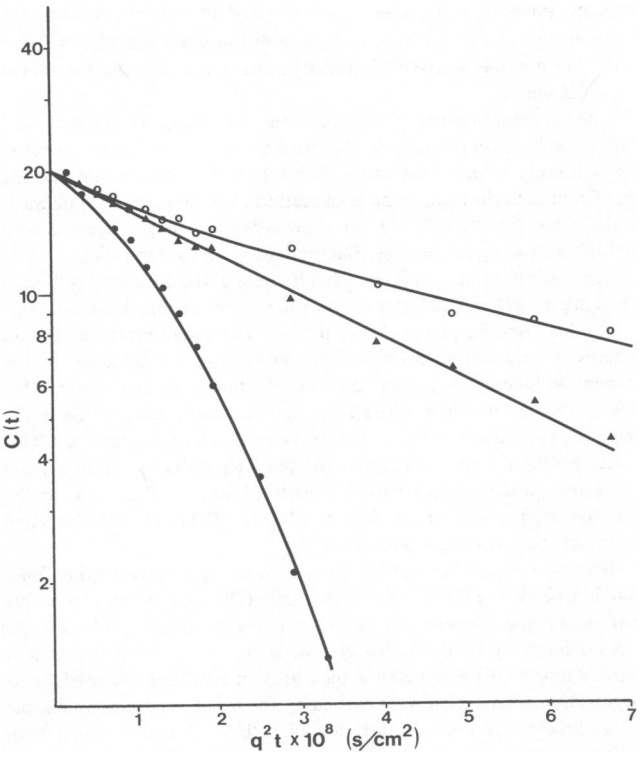

FIGURE 3 A semilog plot of $C(t)$ vs. q^2t for human erythrocytes in PBS + PVP(360) at various concentrations. The scattering angle is 70°. The various concentrations are indicated by different symbols: open circles (○) are for 1.2×10^{-4} (vol/vol), triangles (▲) indicate 2.5×10^{-4} (vol/vol), and filled circles (●) are used for a concentration of 5×10^{-4} (vol/vol).

EXPERIMENTAL RESULTS

According to the hypothesis in reference 2, the existence of the long-range coherent interaction between biological cells requires metabolic activity and the presence of giant dipoles. Additionally, according to the experimental results of Sewchand et al. (15), the presence of a macromolecule is required. Thus a series of experiments were devised that compare the diffusion coefficient of erythrocytes in various combinations of states: metabolically active or inactive, with a normal transmembrane field or without, and in the presence or absence of a suitable macromolecule, PVP(360).

Fig. 4 shows $C(t)$ vs. t for a suspension of erythrocytes in PBS at pH 7.4 and at scattering angles of 60, 70, 80, and 90°. From these data the diffusion coefficient of the erythrocytes is calculated to be 15.6×10^{-10}cm^2/s. Fig. 5 is comparable with Fig. 4 except the suspending medium is PBS + PVP(360). The diffusion coefficient for this case is found to be 16.9×10^{-10}cm^2/s. Fig. 6 is a plot of the decay constant, Γ, vs. q^2 for erythrocytes suspended in PBS + PVP(360). The slope of this line is a measure of the mean diffusion coefficient; its value is 17.0×10^{-10}cm^2/s. Fig. 7 is a plot comparable with Fig. 6 for metabolically inactive erythrocytes. The mean diffusion coefficient is 11.7×10^{-10}cm^2/s.

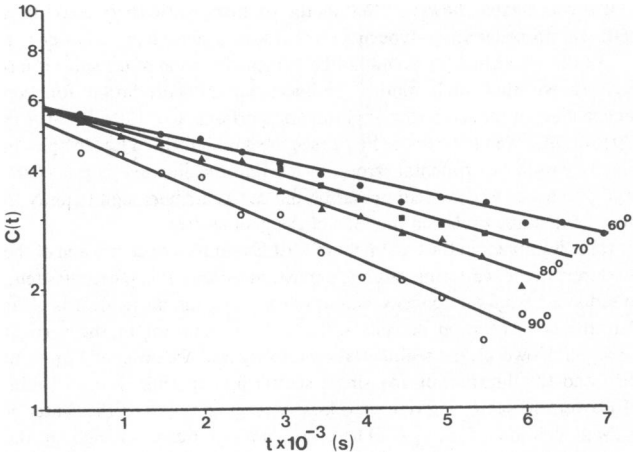

FIGURE 4 $C(t)$ vs. t for human erythrocytes in PBS at pH 7.4 and at various scattering angles.

Data similar to that shown in Figs. 4–7 were gathered under a wide variety of conditions changing both the physiological state of the red blood cell and the parameters of the suspending medium. The results are tabulated in Table II. For convenience in comparing the data a viscosity correction is made: $D_T = D_M\eta_{M,O}$, where D_T is the tabulated diffusion coefficient, D_M is the measured one, and $\eta_{M,O}$ is the viscosity of the suspending medium relative to water as given in Table I. All the values in Table II are the mean values of at least five measurements and are reproducible to within 12%.

In addition to the measurements indicated in Table II, a few others were made that are of interest in this study. PVP(40) and PVP(10) were substituted for PVP(360) as the macromolecule. Microscopic observations showed that neither PVP(10) nor PVP(40) are effective in producing rouleau. The apparent diffusion coefficient of metabolically active erythrocytes at pH 7.4 in PBS + PVP(40) and PBS + PVP(10) were found to be 13.9×10^{-10} and 16.3×10^{-10}cm^2/s, respectively. After a correction is made for the

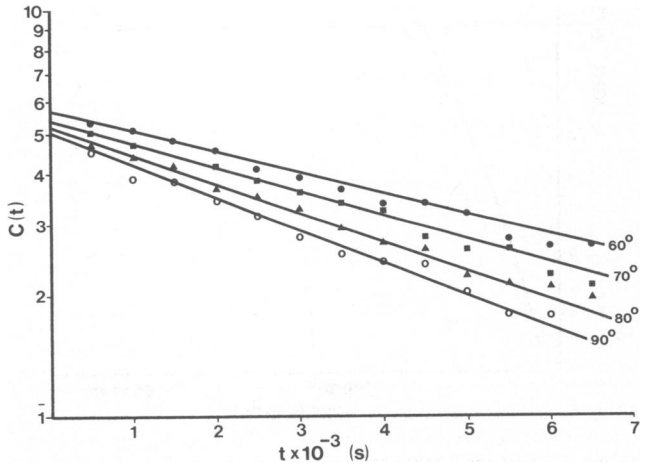

FIGURE 5 $C(t)$ vs. t for human erythrocytes in PBS + PVP(360) at pH 7.4 and at various scattering angles.

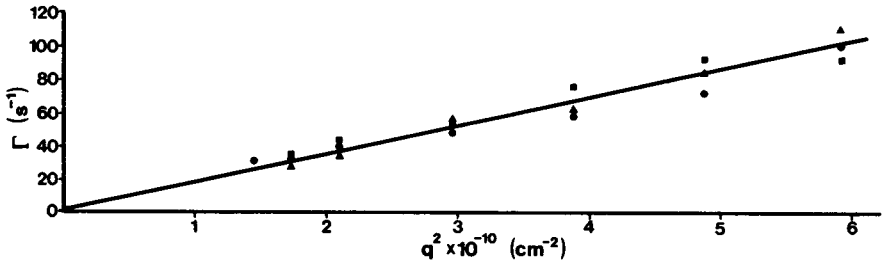

FIGURE 6 The decay constant, Γ, vs. q^2 for normal human erythrocytes in PBS + PVP(360) at pH 7.4. The slope of the line is 17.6 × 10^{-10}cm²/s. The different symbols correspond to different samples of erythrocytes.

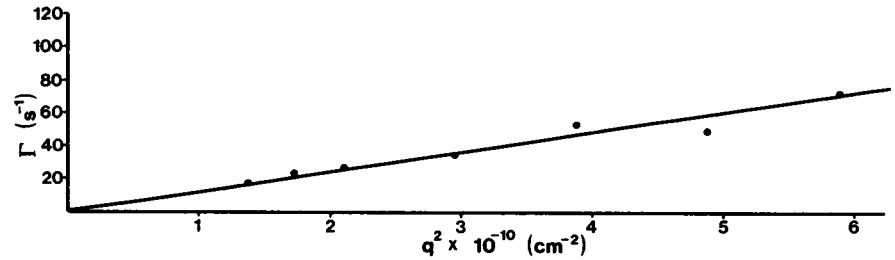

FIGURE 7 The decay constant, Γ, vs. q^2 for metabolically depleted human erythrocytes suspended in PBS + PVP(360) at pH 7.4. The slope of the line is 11.7 × 10^{-10}cm²/s.

relative viscosities of the suspending media, the diffusion coefficients for erythrocytes in PBS + PVP(40) and PBS + PVP(10) are 14.7 × 10^{-10} and 16.8 × 10^{-10} cm²/s, respectively.

These values are not significantly different than those in PBS. The apparent diffusion coefficient of glutaraldehyde-fixed cells was measured in PBS at pH 7.4 and found to be 15.1 × 10^{-10} cm²/s. Glutaraldehyde cross-links the cellular protein, and it is likely that these cells have had their metabolic activity and transmembrane field abolished but their size, shape, and surface charge is unchanged (32).

TABLE II
APPARENT DIFFUSION COEFFICIENTS OF ERYTHROCYTES

	PBS		PBS + PVP (360)			PBS + PVP (40)	PBS + PVP (10)
pH	7.4	6.3	7.4	6.3	7.4(2)	7.4	7.4
Metabolically active	15.9	16.5	23.5	19.5	21.4	14.7	16.8
Metabolically depleted	15.4	14.8	17.5	19.0	18.1	—	—
Metabolically repleted	—	16.2	22.2	19.3	20.6	—	—
Glutaruldehyde fixed	15.1	—	—	—	—	—	—

Diffusion coefficients are given in units of 10^{-10} cm²/s. Metabolically depleted cells refer to cellular suspension that had been incubated for 24 h at room temperature. Metabolically repleted cells are cells that have had their ATP restored (see text). pH 7.4(2) refers to a restoration of the pH of the suspension to 7.4 after a measurement had been made at pH 6.3.

Table II illustrates that the apparent diffusion coefficient of human erythrocytes in phosphate-buffered saline is essentially independent of the pH of the suspending medium and of the metabolic state of the cell. The largest measured variation between differing experimental situations is 11% and the experimental accuracy for any given experiment is 12%. There is a suggestion that at a reduced pH, when the cell is depolarized, the apparent diffusion coefficient is slightly larger for metabolizing cells but the data are not accurate enough to confirm this.

All the values of the measured diffusion coefficient for the erythrocytes in PBS are, within experimental error, the same as for glutaraldehyde-fixed cells in the PBS. The mean value of the apparent diffusion coefficient of human erythrocytes in PBS is 15.8 × 10^{-10} cm²/s.

The situation is quite different when PVP(360) is added to the suspending medium. As seen in Table II there is a wide variation in the measured diffusion coefficient when the metabolic state of the cell is altered or when the pH of the suspending medium is reduced. Clearly, at the normal physiological pH, 7.4, there is a marked reduction in the diffusion coefficient when the metabolic activity of the cell is abolished. Similarly, if the cell is metabolizing normally, there is a reduction in the diffusion coefficient when the pH is reduced to 6.3. These changes are well beyond experimental error and are nearly reversible.

Within experimental error the measured diffusion coefficient is independent of pH when the cell is not metabolizing and is independent of metabolic activity at a reduced pH.

Figs. 8, 9, and 10 are photomicrographs taken through a light microscope at a magnification of either 400 or 1,000.

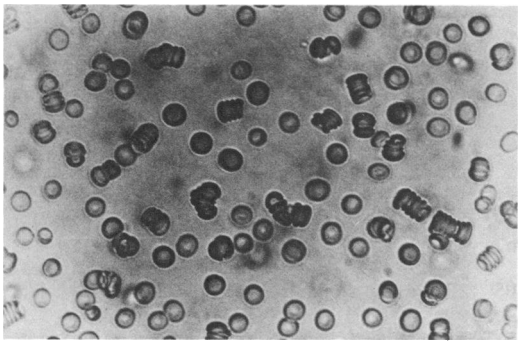

FIGURE 8 Photomicrograph of normal human erythrocytes in PBS + PVP(360) at pH 7.4. The magnification used was 400.

Fig. 8 shows normal cells in PBS + PVP(360) at pH 7.4. As can be seen the shape of the cells is normal and some rouleau have formed. Fig. 9 displays red blood cells in PBS + PVP(360) at a pH 6.3. The cells are nearly normal in shape and a few rouleaux have formed. This is expected since, according to Rowlands et al. (8–10), the rate of formation is not 0 at pH 6.3 but is reduced from the rate observed at pH 7.4.

Metabolically depleted cells are shown in Fig. 10 in a solution of PBS + PVP(360) at pH 7.4. Clearly, metabolic depletion causes crenation of the cell surface. It is also clear that cell-cell adhesion still occurs with these crenated cells but only rarely is a rouleau formed. The diffusion coefficient of the red blood cells measured in this situation is pertinent to this study as it is the rate at which cells come in contact that is of interest. The formation of rouleaux, as opposed to simple conglomerates of cells, apparently depends on the cell shape and other surface parameters of the red blood cells not under consideration in this paper.

DISCUSSION

As mentioned in the Introduction, our main purpose was to obtain data that could be compared with the results of rouleau formation rates. Thus, it is the changes in the apparent diffusion coefficient measured in PBS + PVP(360), as the metabolic activity and the pH are altered, that are of primary interest. However, it is important that one be convinced that what is being measured by these light-scattering experiments is a diffusion coefficient of the red blood cell. The main purpose of the data obtained in PBS is to show that the apparent diffusion coefficients obtained are reasonable and consistent.

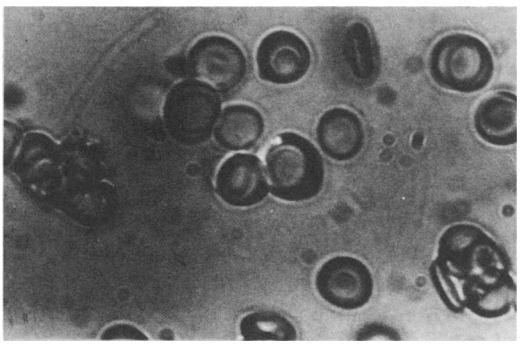

FIGURE 9 Photomicrograph of normal human erythrocytes in PBS + PVP(360) at pH 6.3. The magnification used was 1,000.

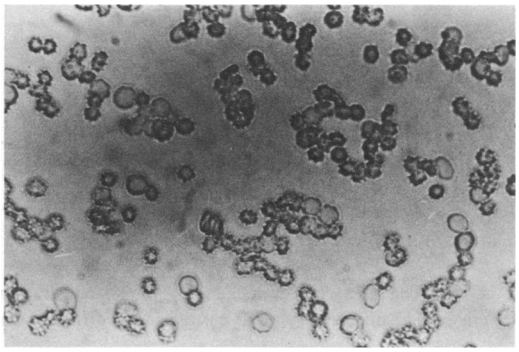

FIGURE 10 Photomicrograph of metabolically depleted human erythrocytes in PBS + PVP(360) at pH 7.4. The magnification used was 400.

A fairly extensive literature search produced only vague references to the diffusion coefficient of red blood cells. Typically found are statements (without references) such as "These values are one to two orders of magnitude greater than the Brownian diffusion coefficient for the red blood cells at the experimental temperature of 15°C" (33) made in reference to a radial dispersion coefficient of between 2×10^{-7} and 1×10^{-8} cm^2/s. The most useful datum was a measurement of the rotational relaxation time of erythrocytes in 0.9% NaCl at a pH 7.4 (34). The value of the rotational relaxation time was found to be 65 s.

Following a treatment by Tanford (35)

$$t_R = 1/2\,\theta, \tag{4}$$

where t_R is the rotational relaxation time and θ is the rotary diffusion coefficient for a sphere

$$\theta = kT/\xi \tag{5}$$

with k and T having their usual significance. ξ is the rotary frictional coefficient given by $\xi = 8\pi\eta R^3$ for spheres of radius R. Using Eqs. 4 and 5 together with the well-known Stokes-Einstein equation, $D = kT/6\pi\eta R$, we have, for spheres,

$$t_R D = (2/3)R^2. \tag{6}$$

Modeling the erythrocyte as an oblate spheroid of semiaxes 4 and 2.5 μm, we calculate an equivalent spherical radius (35) of 3.4 μm. Using this value of R and letting $t_R = 65$ s in Eq. 6, a value of $D = 12 \times 10^{-10}$cm^2/s is found. This is in reasonable agreement with the diffusion coefficients tabulated in Table II for erythrocytes in PBS given the crudeness of approximating the red blood cell as an oblate spheroid. The equivalent spherical radius calculated above can be used to obtain an estimate of the diffusion coeffi-

cient using the Stokes-Einstein relation. The result is a diffusion coefficient of 8.5×10^{-10} cm^2/s, which is within a factor of 2 of the measured values.

It was noted in the discussion of potential sources of artifact that calibration of the apparatus with 8-μm diam spheres yielded results in good agreement with theory. It is felt, therefore, that the light-scattering experiments are measuring an effective diffusion coefficient for the erythrocytes and that the red blood cells are executing Brownian motion regardless of their physiological state when they are suspended in PBS.

The changes in the diffusion coefficients upon abolition of either metabolic activity or the transmembrane potential can be related to the rates of formation of rouleaux measured by Rowlands et al. (8–10) under similar conditions. Chandrasekhar (13) derives a rate constant for coagulation of colloids to be

$$K = 4\pi D_0 R_0 = \frac{2}{3}\frac{kT}{\eta}\frac{R}{r}, \tag{7}$$

where R is the radius of a "sphere of influence" of a single particle of radius r and D_0 is the free-particle diffusion coefficient. In the absence of interparticle interactions $R = r$ and for an attractive potential $R > r$. Placing the effect of the attractive potential in a sphere of influence is arbitrary. The rate constant could equally well be written as

$$K = 4\pi D_{\text{eff}} r \tag{8}$$

where D_{eff} is an effective diffusion coefficient. Equating the two expressions for the rate constant and solving for R/r,

$$\frac{R}{r} = \frac{D_{\text{eff}}}{D_0}. \tag{9}$$

Rowlands et al. (8), following Swift and Friedlander (14), use a rate constant

$$K' = \frac{4}{3}\frac{kT}{\eta}\left\langle\frac{Z}{r}\right\rangle, \tag{10}$$

where Z is an interaction parameter having the dimensions of length. Eqs. 7 and 10 must describe the same phenomenon so

$$\frac{2Z}{r} = \frac{R}{r} = \frac{D_{\text{eff}}}{D_0} \tag{11}$$

if the rate constant and the diffusion coefficient are measured under the same conditions.

From Table 1 of Sewchand et al. (15) the value of Z/r is ~3 for metabolically active human erythrocytes with a normal transmembrane field in PBS + PVP(360). This implies an attractive potential operative between the red blood cells. In the presence of an intercellular potential the diffusion coefficient will no longer be given by the Stokes-Einstein relation but must be rederived taking into account the nonstochastic force the intercellular potential produces.

A general treatment of the dynamics of interacting Brownian particles has been given by Pusey (36,37) and Pusey and Tough (38). They show that in the presence of interparticle interactions two very different time scales are involved in the determination of the mean square displacement of the particles. First, there is the characteristic time, τ_B, related to the solvent forces. This time is defined as $\tau_B = m/f$ where m is the mass of the particle and f is the friction coefficient. For human erythrocytes $\tau_B \sim 2 \times 10^{-6}$s.

Second, there is the characteristic time, τ_I, for the interparticle interaction to change by a significant amount. Following Pusey (37), τ_I can be estimated as "the time taken by a particle to move a reasonable fraction of the mean inter- particle spacing." For the systems under consideration here $\tau_I \sim 30 \times 10^{-3}$s. Since both the rate constant measurements of Rowlands et al. (8) and the diffusion measurements reported in this paper are on a time scale greater than τ_I, the mean square displacement of the erythrocytes may be written as (37, 38)

$$\langle \Delta r_j^2(t) \rangle = 2D_0 t - \frac{D_0 \tau_I t}{f}\left\langle\frac{\delta^2 U(r)}{\delta r_j^2}\right\rangle, \tag{12}$$

where $D_0 = kT/f$, f is the friction coefficient, and $U(r)$ is the interparticle potential. The angular brackets indicate an ensemble average. For a free particle

$$\langle \Delta r_j^2(t) \rangle = 2D_0 t. \tag{13}$$

So, by analogy, we write

$$D_{\text{eff}} = D_0\left[1 - \frac{1}{2}\frac{\tau_I}{f}\left\langle\frac{\delta^2 U(r)}{\delta r_j^2}\right\rangle\right]. \tag{14}$$

Following Fröhlich (1,2), $U(r) = -A/r3$, where A is a parameter of the particular particle, its physiological state, and its environment. Thus

$$\frac{D_{\text{eff}}}{D_0} = 1 + \frac{6\tau_I}{f}\left\langle\frac{A}{r^5}\right\rangle. \tag{15}$$

It can be shown that $A \propto \rho^{-1}$ where ρ is the number density of the particle (Paul, R., R. Chatterjee, and J. A. Tuszynski, manuscript submitted for publication). Hence

$$\frac{D_{\text{eff}}}{D_0} = 1 + \frac{6\tau_I}{f}\left\langle\frac{B}{\rho r^5}\right\rangle. \tag{16}$$

If the mean interparticle separation can be approximated as being proportional to $\rho^{-1/3}$, then

$$\frac{D_{\text{eff}}}{D_0} = 1 + \frac{6\tau_I}{f} B' \rho^{2/3}. \tag{17}$$

Hence

$$\frac{2Z}{r} - 1 = \frac{D_{\text{eff}}}{D_0} - 1 = \frac{6\tau_I}{f} B' \rho^{2/3}. \tag{18}$$

TABLE III
QUANTITATIVE COMPARISON OF THE RESULTS FROM LIGHT-SCATTERING EXPERIMENTS AND MEASUREMENTS OF THE RATE CONSTANT FOR ROULEAUX FORMATION

	$\left(\frac{2Z}{r} - 1\right)^*$	$\left(\frac{D_{eff} - D_o}{D_o}\right)\left(\frac{\rho_R}{\rho_D}\right)^{2/3}$
Metabolically active and transmembrane potential normal	4.8 ± 2.2	5.13 ± 0.87
Metabolically repleted after metabolic depletion with normal transmembrane potential	4.52 ± 1.36	3.71 ± 0.12
Metabolically normal with transmembrane potential abolished (pH 6.3)	1.14 ± 0.82	1.97 ± 0.33
Metabolically normal with transmembrane potential restored (pH 7.4[2])	4.14 ± 1.98	3.25 ± 0.55

D_o refers to the diffusion coefficient measured in PBS. D_{eff} refers to the diffusion coefficient measured in PBS + PVP (360). pH 7.4(2) refers to a restoration of the pH of the suspension to 7.4 after a measurement had been made at pH 6.3.
*See Rowlands et al. (8).

Thus, to quantitatively compare the results of rouleaux formation rates with the diffusion coefficients obtained by light scattering, a correction must be made for the number density of erythrocytes used in the two experiments. Rowlands et al. (8, 9) used a particle concentration of 5×10^{-3} vol/vol, whereas the hematocrit used in this work is 1.8×10^{-4} vol/vol. (Hematocrit equals the volume of a red blood cell times number density.) So $(\rho_R/\rho_D)^{2/3} = 9.17$, where ρ_R refers to the number density used by Rowlands et al. (8, 9) and ρ_D refers to the number density used in the light-scattering experiments.

A quantitative comparison of the results from the two types of experiments is shown in Table III. Note that the numerical agreement is good particularly for the normal cells and the metabolically repleted cells. The agreement is somewhat worse for cells at pH 6.3 and where the pH has been restored to 7.4. This is probably due to unknown dilution factors involved in changing the pH of the suspending medium. It appears, therefore, that both experiments are measuring the same phenomenon; an effect related to an interparticle potential whose existence depends on the simultaneous presence of metabolic activity and a transmembrane potenital.

The fact that a particular macromolecule is required (recall that the diffusion coefficients were normal for both PBS + PVP[40] and PBS + PVP[10]) is curious. The observation that the diffusion coefficient for the erythrocytes is somewhat elevated in PBS + PVP(360), even in the absence of a transmembrane potential or metabolic activity, suggests an interaction between the cell and the macromolecule. This, however, will not account for the relative changes in the diffusion coefficient as the physiological state of the cell is changed in the present of PVP(360).

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