INTERACTION FORCES BETWEEN RED CELLS AGGLUTINATED BY ANTIBODY

I. Theoretical

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ABSTRACT A general method of calculating forces, torques, and translational and rotational velocities of rigid, neutrally buoyant spheres suspended in viscous liquids undergoing a uniform shear flow has been given by Arp and Mason (1977). The method is based on the matrix formulation of hydrodynamic resistances in creeping flow by Brenner and O'Neill (1972). We describe the solution of the Brenner-O'Neill force-torque vector equation in terms of the particle and external flow field coordinates and derive expressions for the normal force acting along, and the shear force acting perpendicular to, the axis of the doublet of spheres, the latter explicitly given for the first time. The equations consist of a term comprising force and torque coefficients obtained from the matrices of the hydrodynamic resistances (functions of the distance h between sphere surfaces which have been computed), and terms comprising the orientation of the doublet axis relative to the coordinates of the external flow field and the shear stress (which can be experimentally determined). We have applied the theory to a system of doublets of sphered, hardened human red cells of group A or B antigenic type cross-linked by the corresponding antibody at a fixed interparticle distance. Working from studies of the breakup of doublets of red cells in an accelerating Poiseuille flow, given in the succeeding paper, we are able to compute the hydrodynamic force required to separate the two spheres. Previous work has shown that the theory can be applied to doublets in a variable shear, Poiseuille flow, provided the ratio of particle to tube diameter is small. In calculating the force-torque coefficients it was assumed that the cells are crosslinked by antibody with h = 20 nm.

INTRODUCTION

The characterization of cell-cell adhesion mechanisms is a fundamental quest in biology. The adhesion of one cell to another is a prerequisite for much of the social life of a cell, both normal and abnormal: fertilization, morphogenesis, proliferation, differentiation, metastasis, and thrombogenesis. Despite its long having been an active area of research, the investigation of adhesion has been impeded by the paucity of appropriate testable models and by methodological and technical difficulties in the experimentation necessary to evaluate such models. In particular there has been the difficulty of generating absolute quantitative data. This paper describes the development of methods in our laboratory to measure the exact force involved in cell-cell adhesion.

In recent years, considerable efforts have been made to develop such experimental methods. Curtis and Hocking, and later, Lerche, have indirectly derived forces of interaction between cells from analysis of the aggregation kinetics of sheared cell suspensions (Curtis, 1969; Curtis, 1970; Curtis and Hocking, 1970; Lerche, 1983). This analytic method is incomplete, as it ignores intercellular repulsive forces. Although several investigators have measured fluid shear stresses as an indication of the average strength of aggregation of a cell suspension, geometric and fluid mechanical considerations have precluded precise quantitation of applied force (Brooks et al., 1970; Schmid-Schönbein et al., 1972; Bongrand et al., 1979). The work of Chien et al. has partly overcome these uncertainties by measuring the hydrodynamic force necessary to disaggregate an individual sessile doublet of discoid erythrocytes (Chien et al., 1977; Chien et al., 1983). They used the bulk fluid shear stress as the disrupting force, which is an approximation of forces acting on such a doublet. Their experimental results were at some variance with their computer simulations of adhesion energy as assessed by membrane curvature. Evans et al. have developed techniques of manipulation of individual cells whereby analysis of their deformation when brought into contact provides a measurement of surface affinity (Evans, 1980; Evans and Buxbaum, 1981). With this technique they have measured the surface affinities of red blood cells that have been agglutinated by plasma and dextrans (Buxbaum et al., 1982). Recently, by micromanipulation, they have measured the force required to disrupt two erythrocytes that

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have been apposed after exposure to wheat germ agglutinin (Evans and Leung, 1984).

The experimental methods of our laboratory consist essentially of the application to biological systems of a theoretically and experimentally well documented corpus from hydrodynamics and colloid chemistry. The behavior of neutrally buoyant, rigid latex spheres of colloidal size (10^{-6} m) interacting in shear flow has been extensively described (van de Ven and Mason, 1976*a*, *b*, 1977; Takamura et al., 1979), as has the behavior of doublets of such spheres when crosslinked by polymer. The work presented here attempts to apply this to a system of doublets of sphered human red blood cells (rbc) of group A or B antigenic type crosslinked by corresponding antibody. The object of the experiments was to measure the hydrodynamic force required to separate the spheres of a doublet. As described in Part II of this paper (Tha et al., 1986), doublets of red cells suspended in aqueous glycerol containing antiserum were tracked in a continuously accelerating flow through a 178-μm tube until breakup. From the experimentally determined shear stress and orientation of the doublet axis at breakup it was possible to calculate the force of separation. In Part I of this work we describe the theory underlying this calculation.

GLOSSARY

Nomenclature

$a, b^*, c, d, e, f^*, g, h^*$	force and torque coefficients of a pair of spheres defined in Eqs. 21 and 22
$A(r^*), C(r^*)$	dimensionless functions of r^* defined in Batchelor and Green (1972) and in van de Ven and Mason (1976*a*)
b	sphere radius
$B(r^*)$	angular velocity coefficient, defined in Eq. 4
C	orbit constant of doublet defined in Eq. 6 of the succeeding paper
D_r	diffusion coefficient for rotation about transverse axis of doublet defined in Eq. 7 of the succeeding paper
f_b	force required to break a single cross-bridge-cell bond
$\mathbf{F}(j); F_i(j); F_i$	force vector acting on sphere j = 1, 2; force components on sphere j along X_i; force acting on center of doublet axis along X_i
$F_{int}(h)$	nonhydrodynamic forces of interaction between two spheres as function of h
F_{sep}	normal or shear force required to separate two cells
F_{shear}	hydrodynamic shear force $= (F_1^2 + F_2^2)^{1/2}$
$\mathbf{G}; G; G(R)$	velocity gradient tensor; shear rate; shear rate as function of radial distance R from tube axis
h	gap distance between sphere surfaces
k	Boltzmann constant
K_r	rotary resistance coefficient
$\ell'(\phi)$	projection of doublet length onto x_2x_3 plane
n	number of cross-bridges between cells
O	origin of particle-fixed coordinate system X_i at center of doublet axis
Pe	Peclet number defined in Eq. 6 of the succeeding paper
$r; r^*$	center-to-center distance of a pair of spheres; r/b
$\mathbf{r}_o$	position vector of a point relative to 0
$r_e(r^*)$	equivalent spheroidal axis ratio of a doublet
R	radial distance from tube axis
R_o	tube radius
$\mathbf{S}, S_{ij}$	rate-of-strain tensor; its components along X_i
T_K	Absolute temperature
$\mathbf{u}; u_i$	undisturbed velocity field vector of uniform shear flow in space-fixed coordinate system; its components along x_i
$u_3(R)$	component of undisturbed velocity field vector along x_i in Poiseuille flow at radial distance R in tube
$U_o; U_i; \mathbf{u}(j); u_i(j)$	undisturbed velocity field vector of uniform shear flow in particle-fixed coordinate system; its components along X_i; undisturbed velocity field vector at sphere center j; its components along X_i
$\mathbf{U}(j); U_i(j)$	translational velocity of sphere j; its components along x_i
$v_3(R)$	particle velocity along x_3 at a radial distance R in tube
x_i, X_i	respective space-fixed and particle-fixed coordinate systems

Greek Symbols

$\alpha_{12}(h), \alpha_3(h)$	coefficients of shear and normal forces as a function of h defined in Eqs. 36 and 37
$\mathbf{\Gamma}(j); \Gamma_i(j); \mathbf{\Gamma}^D; \Gamma_i^D$	torque on sphere j about its center; its components along X_i; torque on a rigid dumbbell about O; its component along X_i
η	viscosity of suspending medium
θ_i, ϕ_i	polar and azimuthal angles relative to the x_i axis
ρ	radius vector of a sphere
ν_D	angular velocity coefficient of a rigid dumbbell defined in Eq. 28
$\omega; \omega_i$	fluid spin vector of the undisturbed uniform fluid flow; its components along X_i
$\mathbf{\Omega}(j); \Omega_i(j)$	angular velocity of sphere j; its components along X_i

Script Symbols

$(\mathcal{F})$	force-torque vector defined in Eq. 10
$(\mathcal{R})$	grand resistance matrix defined in Eq. 10; given in Eq. 21
$(\mathcal{S})$	shear vector of the rate-of-strain tensor S defined in Eq. 10; given in Eq. 17
$(\mathcal{U})$	relative velocity-spin vector of a particle system defined in Eq. 10; given in Eq. 20
(Φ)	shear resistance matrix defined in Eq. 10; given in Eq. 22

EQUATIONS OF MOTION

The fluid mechanical problem of predicting the trajectories of two interacting, neutrally buoyant spherical particles of equal size suspended in a Newtonian fluid undergoing simple shear flow has been solved (Lin et al., 1970; Batchelor and Green, 1972; Arp and Mason, 1977), including the case when interaction forces, $F_{int}(h)$, other than hydrodynamic operate as sphere surfaces approach to within a distance $h < 100$ nm (van de Ven and Mason, 1976*a*). Assuming that the interaction forces act along the line joining the centers of the spheres, it has been shown that the relative velocity of the centers separated by a distance r is given by (van de Ven and Mason, 1976*a*):

$$\frac{dr}{dt} = A(r^*)Gb\sin^2\theta_1 \sin 2\phi_1 + \frac{C(r^*)F_{\mathrm{int}}(h)}{3\pi\eta b}, \quad (1)$$

and the angular motion of the doublet axis by:

$$\frac{d\theta_1}{dt} = \frac{1}{4}GB(r^*)\sin 2\theta_1 \sin 2\phi_1, \quad (2)$$

$$\frac{d\theta_1}{dt} = \frac{1}{2}G[1 + B(r^*)\cos 2\theta_1]. \quad (3)$$

Here, G is the shear rate, b the particle radius, η the suspending medium viscosity, θ_1 and ϕ_1 the respective polar and azimuthal angles relative to x_1, the vorticity axis, as shown in Fig. 1 *a* for a two-body collision in Poiseuille flow. $A(r^*)$ and $C(r^*)$ are known dimensionless functions of $r^* = r/b$, and $B(r^*)$ is the angular velocity coefficient (Bretherton, 1962) related to the equivalent ellipsoidal axis ratio of the doublet, $r_e(r^*)$ by:

$$B(r^*) = \frac{r_e^2(r^*) - 1}{r_e^2(r^*) + 1}. \quad (4)$$

No complete analytic expression exists for $r_e(r^*)$; asymptotic expressions for large and small r^*, and tabulated values are found in Arp and Mason (1977).

Rearrangement of Eq. 1 yields the force equation:

$$\frac{3\pi\eta b}{C(r^*)}\frac{dr}{dt} = \frac{A(r^*)}{C(r^*)}3\pi\eta G b^2 \sin^2\theta_1 \sin 2\theta_1 + F_{\mathrm{int}}(h). \quad (5)$$

The term on the left represents the hydrodynamic drag force resisting approach of the particles. The first term on the right is the normal hydrodynamic force between the spheres acting along the line of their centers = F_3,[1] and is maximal for $\theta_1 = \pi/2$, i.e., rotation limited to the x_2x_3-plane (Fig. 1 *b*), at $\phi_1 = -\pi/4$, where the force is compressive, and at $\phi_1 = \pi/4$, where it is tensile (Fig. 1 *b*). Eqs. 1–3 have been shown to apply in Poiseuille flow, providing the ratio of particle to tube radius, $b/R_0 \ll 1$ (Takamura et al., 1979, 1981*a*).

In the absence of interaction forces, $F_{\mathrm{int}}(h) = 0$, the trajectories of approach and recession of the spheres would be symmetrical about $\phi_1 = 0$, as shown in Fig. 1 *a*. In the case of doublets of charged latex spheres, $F_{\mathrm{int}}(h) \neq 0$, owing to double layer electrostatic repulsion and van der Waals attraction, and the trajectories are no longer symmetrical. Some collisions result in the formation of permanent doublets, the spheres of which are in a secondary potential energy minimum at distances h between 15 and 17 nm and are capable of independent rotation as shown by measurements of the period of rotation, T, of the doublets about 2π (Takamura et al., 1979). The period T is obtained by integration of Eq. 3, giving the angular velocity of a rigid prolate ellipsoid (Jeffery, 1922) of equivalent axis ratio $r_e(r^*)$:

$$\tan\phi_1 = r_e(r^*)\tan\frac{2\pi t}{T}, \quad (6)$$

where T is defined by:

$$T = \frac{2\pi}{G}[r_e(r^*) + r_e^{-1}(r^*)]. \quad (7)$$

It has been shown that the dimensionless period of rotation, TG, for doublets of nontouching spheres depends not only on the distance of separation of the sphere surfaces, but also on whether they are capable of rotation relative to each other (Arp and Mason, 1977); $r_e(r^*)$ has a different solution for each of these cases. TG at a given r^*

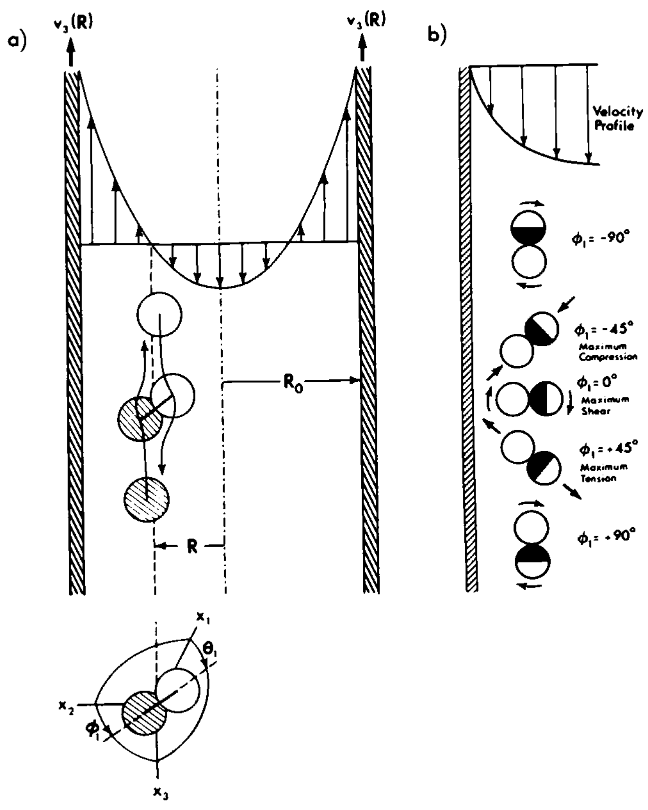

FIGURE 1 (*a*) Two-body collision between rigid spheres in Poiseuille flow showing the formation of a transient doublet in the median plane of the tube of radius R_0 which is moved upward with a velocity $v_3(R)$, equal to that of the downward flowing doublet whose center is at a radial distance R from the axis. Cartesian (x_i) and spherical polar coordinates (ϕ_1, θ_1) are constructed at the midpoint of the doublet axis. (After Takamura et al., 1979). (*b*) Rotation over ½ orbit of a permanent doublet of rigid spheres in Poiseuille flow with $\theta_1 = 90°$, showing the ϕ_1-orientations corresponding to maximum normal and shear stress.

[1]The subscript 3 refers to the particle coordinate X_3, as shown in Fig. 2 and described below.

is markedly greater for independently rotating than for rigidly coupled spheres. The experimental determination of *TG* is therefore a sensitive indicator of a doublet being rigidly linked.

In the presence of cationic polyelectrolyte, permanent doublets are formed which rotate as dumbbells of rigidly linked spheres, presumably because polymer bridge formation has occurred (Takamura et al., 1979, 1981*b*). In the case of permanent doublets of red cells, the spheres are linked by antigen-antibody bridges; no assumptions need be made as to the characteristics of the linkage as $h/b \ll 1$ (Adler et al., 1981) other than it rigidly link the spheres such that no independent motion is possible.[2] For such a doublet in shear flow, $dr/dt = 0$, and if it can be induced to break up, then:

$$F_{\mathrm{int}}(h) = -F_3 = -\frac{A(r^*)}{C(r^*)} 3\pi\eta G b^2 \sin^2\theta_1 \sin 2\phi_1. \quad (8)$$

However, the bonds linking the red cell spheres are not only subject to hydrodynamic forces acting along the doublet axis, but also normal to it; i.e., shear forces.

The following derives the equations for calculating the normal and the shear forces acting on a doublet whose axis may lie in any plane.

FORCES ACTING BETWEEN SPHERES

Formulation of the Problem

We consider a doublet of equal-sized, rigid spheres of radius b which rotates as a rigidly-linked dumbbell in a linear shear field[3] with origin O at the center of the doublet axis, as shown in Fig. 2. The doublet axis has length $r = (2b + h)$, where h is the gap distance between sphere surfaces. The space-fixed coordinates, x_i, of the shear field are: x_1, the vorticity axis, x_2, the direction of the velocity gradient, and x_3, the direction of the undisturbed flow. Here,

$$u_1 = u_2 = 0; \quad u_3 = Gx_2, \quad (9)$$

where u_i are components of the undisturbed velocity field vector $\mathbf{u}$ and G is the shear rate. The particle-fixed coordinates X_i have origin O at the center of the doublet axis, where X_3 lies along the doublet axis, X_2 is coplanar with the x_1x_3-plane, and X_1 is perpendicular to X_2 and X_3.

Under creeping flow conditions, the force vector $\mathbf{F}(j)$ and the torque vector $\mathbf{\Gamma}(j)$ acting respectively at and about the sphere $j = 1, 2$ centers of the doublet can be expressed in terms of X_i coordinates by the general formulation of

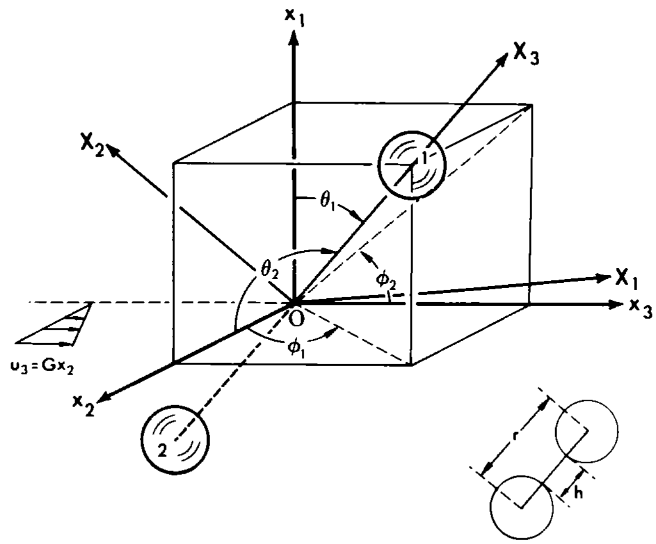

FIGURE 2 Particle (X_i) and external shear field (x_i) coordinates used to describe the rotation, with angular velocity $\mathbf{\Omega}$, of a doublet of rigidly linked spheres whose center, O, is located at the origin of a field of Couette flow, $u_3 = Gx_2$: x_3 is the direction of the undisturbed flow, x_2 is the direction of shear, and x_1 is the direction of vorticity. The particle coordinate X_3 is directed from O to the center of sphere (1), X_2 is coplanar with the x_1x_3-plane, and X_1 is perpendicular to X_2 and X_3. As shown at lower right, sphere centers are separated by a distance r, and sphere surfaces by a distance h.

Brenner and O'Neill (1972):

$$(\mathcal{F}) = \begin{pmatrix} \mathbf{F}(1) \\ \mathbf{F}(2) \\ \mathbf{\Gamma}(1) \\ \mathbf{\Gamma}(2) \end{pmatrix} = -\eta\{(\mathcal{R})(\mathcal{U}) + (\Phi)(\mathcal{S})\}. \quad (10)$$

where $(\mathcal{F})$ is the force-torque vector, $(\mathcal{R})$ the grand resistance matrix, (Φ) the shear resistance matrix; $(\mathcal{S}) = (S_{11}, S_{22}, S_{33}, S_{23}, S_{13}, S_{12})$ is the shear vector of the rate-of-strain tensor $\mathbf{S}$, and $(\mathcal{U})$ is the relative velocity-spin vector:

$$(\mathcal{U}) = \begin{pmatrix} \mathbf{U}(1) - \mathbf{u}(1) \\ \mathbf{U}(2) - \mathbf{u}(2) \\ \mathbf{\Omega}(1) - \omega \\ \mathbf{\Omega}(2) - \omega \end{pmatrix} \quad (11)$$

where $\mathbf{U}(1)$ is the translational velocity of sphere (1), $\mathbf{u}(1)$ is the undisturbed velocity field vector at sphere (1) center, $\mathbf{\Omega}(1)$ the angular velocity of sphere (1), and ω the fluid spin vector of the undisturbed uniform fluid flow; and likewise for sphere (2). Since the values of $(\mathcal{R})$ and (Φ) are known, the solution rests on deriving equations for $(\mathcal{U})$ and $(\mathcal{S})$. Advantage may be taken of the symmetry properties of equal-sized spheres rotating as a doublet in uniform shear flow, as they experience equal but opposite forces and equal torques. Likewise, the spheres move with equal but opposite translational velocities and equal rotational veloci-

[2]The validity of this assumption is shown in Part II of this paper.

[3]Although this treatment strictly applies to a linear shear field, the velocity gradient in Poiseuille flow may be considered to be constant over the radial distance occupied by a doublet in our experiments, <8% of the tube diameter.

ties (Arp and Mason, 1977); therefore consideration of sphere (1) alone suffices to establish the dynamics of the pair.

Calculation of Vectors and Matrices

To calculate the components of $\mathbf{F}(1)$ and $\mathbf{\Gamma}(1)$, which are related to the particle-fixed coordinate system, it is necessary to establish the transformation law between the unit vector system $\mathbf{i}_i$ of the space-fixed coordinates, and the unit vector system, $\mathbf{e}_i$ of the particle-fixed coordinates (Arp and Mason, 1977):

$$\begin{pmatrix}\mathbf{i}_1\\ \mathbf{i}_2\\ \mathbf{i}_3\end{pmatrix} = \begin{pmatrix}\cos\theta_2\sin\phi_2 & \cos\phi_2 & \sin\theta_2\sin\phi_2\\ -\sin\theta_2 & 0 & \cos\theta_2\\ \cos\theta_2\cos\phi_2 & -\sin\phi_2 & \sin\theta_2\cos\phi_2\end{pmatrix}\cdot\begin{pmatrix}\mathbf{e}_1\\ \mathbf{e}_2\\ \mathbf{e}_3\end{pmatrix} \quad (12)$$

Here, θ_2 and ϕ_2 are the Euler angles for rotating $\mathbf{e}_i$ into $\mathbf{i}_i$, and are also the respective polar and azimuthal angles referred to x_2 as the polar axis (Fig. 2). Hence:

$$\begin{aligned} x_1 &= X_1\cos\theta_2\sin\phi_2 + X_2\cos\phi_2 + X_3\sin\theta_2\sin\phi_2\\ x_2 &= -X_1\sin\theta_2 + X_3\cos\theta_2\\ x_3 &= X_1\cos\theta_2\cos\phi_2 - X_2\sin\phi_2 + X_3\sin\theta_2\cos\phi_2 \quad (13)\end{aligned}$$

The undisturbed flow having components u_i in x_i (Eq. 9) is a vector field $\mathbf{U}_o$ having components U_i in X_i:

$$\begin{aligned} U_1 &= u_3\cos\theta_2\cos\phi_2 = G(-X_1\sin\theta_2\cos\theta_2\cos\phi_2\\ &\quad + X_3\cos^2\theta_2\cos\phi_2)\\ U_2 &= -u_3\sin\phi_2 = G(X_1\sin\theta_2\sin\phi_2 - X_3\cos\theta_2\sin\phi_2)\\ U_3 &= u_3\sin\theta_2\cos\phi_2 = G(-X_1\sin^2\theta_2\cos\phi_2\\ &\quad + X_3\sin\theta_2\cos\theta_2\cos\phi_2) \quad (14)\end{aligned}$$

The velocity gradient tensor $\mathbf{G}$, where $\mathbf{G} = \nabla\mathbf{U}_o$, is here in terms of X_i:

$$\mathbf{G} = \begin{pmatrix}-G\sin\theta_2\cos\theta_2\cos\phi_2 & 0 & G\cos^2\theta_2\cos\phi_2\\ G\sin\theta_2\sin\phi_2 & 0 & -G\cos\theta_2\sin\phi_2\\ -G\sin^2\theta_2\cos\phi_2 & 0 & G\sin\theta_2\cos\theta_2\cos\phi_2\end{pmatrix} \quad (15)$$

and the rate-of-strain tensor $\mathbf{S} = \tfrac{1}{2}(\mathbf{G} + \mathbf{G}\dagger)$ is:

$$\mathbf{S} = \begin{pmatrix}-\tfrac{1}{2}G\sin 2\theta_2\cos\phi_2 & \tfrac{1}{2}G\sin\theta_2\sin\phi_2 & \tfrac{1}{2}G\cos 2\theta_2\cos\phi_2\\ \tfrac{1}{2}G\sin\theta_2\sin\phi_2 & 0 & -\tfrac{1}{2}G\cos\theta_2\sin\phi_2\\ \tfrac{1}{2}G\cos 2\theta_2\cos\phi_2 & -\tfrac{1}{2}G\cos\theta_2\sin\phi_2 & \tfrac{1}{2}G\sin 2\theta_2\cos\phi_2\end{pmatrix} \quad (16)$$

Hence, the shear vector $(\mathscr{S})$ is:

$$(\mathscr{S}) = \begin{pmatrix}-\tfrac{1}{2}G\sin 2\theta_2\cos\phi_2\\ 0\\ \tfrac{1}{2}G\sin 2\theta_2\cos\phi_2\\ -\tfrac{1}{2}G\cos\theta_2\sin\phi_2\\ \tfrac{1}{2}G\cos 2\theta_2\cos\phi_2\\ \tfrac{1}{2}G\sin\theta_2\sin\phi_2\end{pmatrix} \quad (17)$$

It now remains to determine $(\mathscr{U})$ (Eq. 11). In tensor notation, the undisturbed velocity field vector $\mathbf{u}$ and undisturbed fluid spin vector $\boldsymbol{\omega}$ may be written as (Arp and Mason, 1977):

$$\mathbf{u} = \mathbf{r}_o\cdot\mathbf{G} = G\mathbf{r}_o\cdot(\mathbf{i}_2\mathbf{i}_3), \quad (18a)$$

$$\boldsymbol{\omega} = \tfrac{1}{2}G\mathbf{i}_1, \quad (18b)$$

where $\mathbf{r}_o$ is the position vector of a point relative to 0, the origin of the flow field. From Eqs. 18 and 12 it follows that:

$$\mathbf{u} = \tfrac{1}{2}Gr(\cos^2\theta_2\cos\phi_2 \quad -\cos\theta_2\sin\phi_2 \quad \tfrac{1}{2}\sin 2\theta_2\cos\phi_2) \quad (19a)$$

$$\boldsymbol{\omega} = \tfrac{1}{2}G(\cos\theta_2\sin\phi_2 \quad \cos\phi_2 \quad \sin\theta_2\sin\phi_2) \quad (19b)$$

For a rigidly-linked dumbbell, the spheres of which are unable to undergo relative rotation, the components of $U(j)$ are: $U_1(j) = \tfrac{1}{2}r\Omega_2(j)$, $U_2(j) = -\tfrac{1}{2}r\Omega_1(j)$, and $U_3(j) = 0$. Since it is known that $\Omega_3(j) = \tfrac{1}{2}G\sin\theta_2\sin\phi_2$ (Jeffery, 1922), $\Omega_3(1) - \omega_3 = 0$. Hence, $(\mathscr{U})$ for sphere (1) is, from Eq. 11:

$$(\mathscr{U}) = \begin{pmatrix}\tfrac{1}{2}\Omega_2 r - \tfrac{1}{2}Gr\cos^2\theta_2\cos\phi_2\\ -\tfrac{1}{2}\Omega_1 r + \tfrac{1}{2}Gr\cos\theta_2\sin\phi_2\\ -\tfrac{1}{2}Gr\sin 2\theta_2\cos\phi_2\\ \Omega_1 - \tfrac{1}{2}G\cos\theta_2\sin\phi_2\\ \Omega_2 - \tfrac{1}{2}G\cos\phi_2\\ 0\end{pmatrix} \quad (20)$$

Finally, $(\mathscr{R})$ and (Φ) for sphere (1) are (Brenner and O'Neill, 1972):

$$(\mathscr{R}) = \begin{bmatrix}a & 0 & 0 & 0 & -c & 0\\ 0 & a & 0 & c & 0 & 0\\ 0 & 0 & b^* & 0 & 0 & 0\\ 0 & c & 0 & d & 0 & 0\\ -c & 0 & 0 & 0 & d & 0\\ 0 & 0 & 0 & 0 & 0 & e\end{bmatrix} \quad (21)$$

and

$$(\Phi) = \begin{bmatrix}0 & 0 & 0 & 0 & 2g & 0\\ 0 & 0 & 0 & 2g & 0 & 0\\ 0 & 0 & f^* + 2g & 0 & 0 & 0\\ 0 & 0 & 0 & 2h^* & 0 & 0\\ 0 & 0 & 0 & 0 & -2h^* & 0\\ 0 & 0 & 0 & 0 & 0 & 0\end{bmatrix} \quad (22)$$

where a, b^*, c, d, e, f^*, g, h^* are force and torque coefficients for sphere (1) that are known and tabulated functions of h and of b.[4]

[4]Note that Eq. 21 differs from that given by Arp and Mason (1977) in that the signs of the coefficients of c are reversed.

Calculation of Shear and Normal Force

The shear force acting on each sphere of the doublet is given by:

$$F_{\text{shear}} = (F_1^2 + F_2^2)^{1/2}. \quad (23)$$

Evaluating the F_1 component of Eq. 10 with the aid of Eqs. 17 and 20–22 yields:

$$F_1(1) = -\eta\{\tfrac{1}{2}ar(\Omega_2 - G\cos^2\theta_2\cos\phi_2) - c(\Omega_2 - \tfrac{1}{2}G\cos\phi_2) + gG\cos 2\theta_2\cos\phi_2\}. \quad (24)$$

In order to solve for Ω_2, advantage is taken of the absence of torques about the center of the doublet axis (Arp and Mason, 1977):

$$\Gamma_2^{\text{D}} = rF_1(1) + 2\Gamma_2(1) = 0, \quad (25)$$

where Γ_2^{D} is the component along X_2 of $\boldsymbol{\Gamma}^{\text{D}}$, the torque on a rigid dumbbell about the origin O; and $\Gamma_2(1)$ is the component along X_2 of $\Gamma(1)$, the torque on sphere (1) about its center. The component $\Gamma_2(1)$ of Eq. 10 is:

$$\Gamma_2(1) = -\eta\{\tfrac{1}{2}cr(\Omega_2 - G\cos^2\theta_2\cos\phi_2) + d(\Omega_2 - \tfrac{1}{2}G\cos\phi_2) - h^*G(\cos 2\theta_2\cos\phi_2)\}. \quad (26)$$

The solution of Eq. 26 for Ω_2 is:

$$\Omega_2 = \tfrac{1}{2}G(-\nu_{\text{D}}\cos 2\theta_2\cos\phi_2 + \cos\phi_2) = -\nu_{\text{D}}S_{13} + \omega_2, \quad (27)$$

where $\nu_{\text{D}} = -\text{B}(r^*)$ (Eq. 4) is the angular velocity coefficient of the doublet, given by (Arp and Mason, 1977):

$$\nu_{\text{D}} = \frac{-\tfrac{1}{2}ar^2 + cr + 2rg + 4h^*}{\tfrac{1}{2}ar^2 - 2rc + 2d}. \quad (28)$$

Substituting for Ω_2 in Eq. 24 finally yields:

$$F_1(1) = -\tfrac{1}{2}\eta G\{\nu_{\text{D}}(-\tfrac{1}{2}ar + c) - \tfrac{1}{2}ar + 2g\}\cos 2\theta_2\cos\phi_2. \quad (29)$$

Similarly, for F_2, using the equality

$$\Gamma_1^{\text{D}} = -rF_2(1) + 2\Gamma_1(1) = 0, \quad (30)$$

$$F_2(1) = -\eta\{\tfrac{1}{2}ar(-\Omega_1 + G\cos\theta_2\sin\phi_2) + c(\Omega_1 - \tfrac{1}{2}G\cos\theta_2\sin\phi_2) + gG\cos\theta_2\sin\phi_2\} \quad (31a)$$

$$\Gamma_1(1) = -\eta\{\tfrac{1}{2}cr(-\Omega_1 + G\cos\theta_2\sin\phi_2) + d(\Omega_1 - \tfrac{1}{2}G\cos\theta_2\sin\phi_2) + h^*G\cos\theta_2\sin\phi_2\} \quad (31b)$$

whence,

$$\Omega_1 = \tfrac{1}{2}G(-\nu_{\text{D}} + 1)\cos\theta_2\sin\phi_2 = \nu_{\text{D}}S_{23} + \omega_1 \quad (32)$$

and,

$$F_2(1) = -\tfrac{1}{2}\eta G\{\nu_{\text{D}}(\tfrac{1}{2}ar - c) + \tfrac{1}{2}ar - 2g\}\cos\theta_2\sin\phi_2. \quad (33)$$

The magnitude of F_{shear}, i.e., forces perpendicular to the doublet axis is then:

$$(F_1^2 + F_2^2)^{1/2} = -\tfrac{1}{2}\eta G\{\nu_{\text{D}}(-\tfrac{1}{2}ar + c) - \tfrac{1}{2}ar + 2g\} \times \{(\cos 2\theta_2\cos\phi_2)^2 + (\cos\theta_2\sin\phi_2)^2\}^{1/2}. \quad (34)$$

The normal force acting on sphere (1) is given by $F_3(1)$, which from Eqs. 10, 17, and 20–22 is:

$$F_3(1) = -\tfrac{1}{2}\eta G(-\tfrac{1}{2}b^*r + f^* + 2g)\sin 2\theta_2\cos\phi_2, = -\tfrac{1}{2}\eta G(-\tfrac{1}{2}b^*r + f^* + 2g)\sin^2\theta_1\sin 2\phi_1.^5 \quad (35)$$

When the doublet rotates exclusively in the x_2x_3-plane, i.e., $\theta_1 = \pi/2$, $\phi_2 = 0$ and $\theta_2 = \phi_1$ (Fig. 2), Eqs. 34 and 35 reduce to:

$$F_1(1) = -\tfrac{1}{2}\eta G\{(\nu_{\text{D}}(-\tfrac{1}{2}ar + c) - \tfrac{1}{2}ar + 2g)\cos 2\phi_1\}, \quad (36)$$

$$F_3(1) = -\tfrac{1}{2}\eta G\{(-\tfrac{1}{2}b^*r + f^* + 2g)\sin 2\phi_1\}, \quad (37)$$

and as $F_2(1)$ here $= 0$, $F_1(1) = F_{\text{shear}}$.

Eqs. 34 and 35 can be written in the form:

$$(F_1^2 + F_2^2)^{1/2} = \alpha_{12}(h)\eta G\text{b}^2\{(\cos 2\theta_2\cos\phi_2)^2 + (\cos\theta_2\sin\phi_2)^2\}^{1/2} \quad (38)$$

$$F_3(1) = \alpha_3(h)\eta G\text{b}^2\sin^2\theta_1\sin 2\phi_1. \quad (39)$$

where α_{12} and α_3 are force coefficients, functions of h, obtained by factoring out b^2 from the matrix coefficients.

FORCES ACTING BETWEEN RED CELLS

The treatment so far applies to all undeformable spheres linked rigidly by any material. In our model of antibody-mediated hemagglutination, we are dealing with sphered red cells bonded by discrete molecular crossbridges of specific antibody. It is therefore necessary to make an estimate of h for doublets of red cells, the force coefficients varying by $\leq$7% over the range $0 < h < 1$ μm. The thickness of the red cell glycocalyx is uncertain; we have taken it as 7.5 nm as an average from the literature (Levine et al., 1983). The diameter of IgM is 30 nm (Feinstein and Munn, 1969). Therefore, the maximum h permitting any linkage between the membranes of the spheres $\simeq$45 nm. Calculations from modeling suggest that spacing between lipid bilayers of erythrocytes (in the absence of polymer) of $\geq$20 nm will be energetically favored (Parsegian and Gingell, 1972; van Oss and Absolom, 1983; Bongrand and Bell, 1984). The supposition that bonding occurs at separations between 20 and 45 nm is supported by measurements of fibrinogen- and dextran-linked erythrocytes from electron micrographs. For planar membranes, these reveal separations of 22–30 nm for the high molecular weight fractions of dextran (Chien and Jan, 1973). For our system, we have chosen a value of $h = 20$ nm, which leads

[5] Using the relations $\sin\theta_2\cos\phi_2 = \sin\theta_1\sin\phi_1$, $\cos\theta_2 = \sin\theta_1\cos\phi_1$ and $\cos\theta_1 = \sin\theta_2\sin\phi_2$.

to values of the force coefficients $\alpha_{12} = 7.02$ and $\alpha_3 = 19.33$. Thus the equations for shear and normal forces are, from Eqs. 23, 38, and 39:

$$F_{\text{shear}} = 7.02\eta G b^2 \{(\cos 2\theta_2 \cos \phi_2)^2 + (\cos \theta_2 \sin \phi_2)^2\}^{1/2} \quad (40)$$

$$F_3 = 19.33\eta G b^2 \sin^2 \theta_1 \sin 2\phi_1. \quad (41)$$

The ratio of F_3 to F_{shear} is usually close to, or the same as that of their coefficients, 2.75.

The spherical geometry imposes a maximum on the area in which bonding can occur, and therefore on the number of crossbridges possible. To determine the maximum area of interaction we reason that if the maximum separation permitting bonding $\simeq$45 nm, and the closest approach is 20 nm, then all parts of the surface area of the two spheres within 45 nm of each other can potentially interact in bond formation. For $h \simeq 20$ nm, this specifies any part of a single sphere within 12.5 nm of a plane tangent to the point of nearest approach, i.e., tangent to the point of intersection of the doublet axis with the cell membrane. The surface area of a sphere of radius b is given by: $2\pi b \int_{-b}^{b} d\rho$, ρ being the radius vector. Setting $b = 3.21$ μm and resetting the lower limit to $b - 12.5$ nm $= (3.21 - 0.0125)$ μm, integration shows the maximum area over which bonding can be present, $\simeq 0.25$ μm^2. Given A and B antigen densities on the order of 10^6/cell (Economidou et al., 1967), the maximum number of potential bonding sites in this area is $\approx$2,000. Because of steric considerations, the actual number of crossbridges forming would likely be much lower, especially at low antibody concentrations. The minimum area of contact is, potentially, of molecular dimensions, i.e., one molecular crossbridge. The existence of one molecular bond has been surmised to be sufficient to initiate cell adhesion by lectins (Capo et al., 1982) which are energetically comparable to antibodies.

The geometry also imposes the condition of simultaneous breakage of bonds. Whether the shear or normal force is responsible for doublet separation is moot. The normal force must break all bonds simultaneously to effect separation; however, there being no moment about the contact ($\Gamma^D = 0$), this constraint also obtains for separation by shear forces. As discussed by Bell (1978), inasmuch as the antibodies are free to orient themselves relative to the sphere surfaces, the shear force at the contact may be turned into stresses on the individual bonds that are tensile. With simultaneous bond breakage, the force of separation for two cells will be a function of the number of crossbridges and the strength of each cross-bridge:

$$F_{\text{sep}} = n \cdot f_b, \quad (42)$$

where F_{sep} represents either the normal or shear force, n the number of crossbridges between cells, and f_b the force necessary to rupture an individual crossbridge–cell bond. In principle, our model at low n allows the assessment of f_b.

CONCLUDING REMARKS

We have given equations for the hydrodynamic force acting along and perpendicular to the axis of a doublet of rigid spheres. The equations were derived from existing fluid-mechanical theory applicable to doublets rotating in a uniform shear field (Couette flow, Eq. 9) in the absence of inertial effects and particle sedimentation. The spheres were assumed to be rigidly linked, i.e., incapable of relative translational or rotational motion so that the hydrodynamic force required to break up the doublet is equal to the force of interaction between the particles (Eq. 8). Provided the particle to tube diameter ratio is small (<8% in our experiments), the equations have been shown to apply in Poiseuille flow (Takamura et al., 1979, 1981*a*). Inertial effects are negligible since maximum linear fluid velocities were <900 $\mu m\ s^{-1}$, corresponding to tube Reynolds numbers in the least viscous suspending fluid $<5 \times 10^{-3}$.

The succeeding paper describes the application of the theory to calculate the shear and normal hydrodynamic forces necessary to disrupt the antibody-mediated adhesion of two sphered, glutaraldehyde-fixed red blood cells from experiments in which the doublets were subjected to a uniformly accelerating Poiseuille flow until breakup. The remaining assumption, that the red cells are rigidly linked, will be tested by measurements of the dimensionless period of rotation, TG, of the doublets as described in Part II of this paper.

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REFERENCES

Adler, P. M., K. Takamura, H. L. Goldsmith, and S. G. Mason. 1981. Particle motions in sheared suspensions. XXX. Rotations of rigid and flexible dumbbells (theoretical). *J. Colloid Interface Sci.* 83:502–515.

Arp, P. A., and S. G. Mason. 1977. The kinetics of flowing dispersions. VIII. Doublets of rigid spheres (theoretical). *J. Colloid Interface Sci.* 61:21–43.

Batchelor, G. K., and J. T. Green. 1972. The hydrodynamic interaction of two small freely moving spheres in a linear flow field. *J. Fluid Mech.* 56:375–400.

Bell, G. I. 1978. Models for the specific adhesion of cells to cells. *Science (Wash. D.C.).* 200:618–627.

Bongrand, P., and G. I. Bell. 1984. Cell-cell adhesion: parameters and possible mechanisms. *In* Cell Surface Dynamics: Concepts and Models. C. DeLisi, A. Perelson, and F. Wiegel, editors. Marcel Dekker, Inc., New York. 459–493.

Bongrand, P., C. Capo, A.-M. Benoliel, and R. Despieds. 1979. Evaluation of intercellular adhesion with a very simple technique. *J. Immunol. Methods.* 28:133–141.

Brenner, H., and M. E. O'Neill. 1972. On the Stokes resistance of multiparticle systems in a linear shear field. *Chem. Eng. Sci.* 27:1421–1439.

Bretherton, F. P. 1962. The motion of rigid particles in a shear flow at low Reynolds number. *J. Fluid Mech.* 14:284–304.

Brooks, D. E., J. W. Goodwin, and G. V. F. Seaman. 1970. Interactions among erythrocytes under shear. *J. Appl. Physiol.* 28:172–177.

Buxbaum, K., E. A. Evans, and D. E. Brooks. 1982. Quantitation of surface affinities of red blood cells in dextran solutions. *Biochemistry.* 21:3235–3239.

Capo, C., F. Garrouste, A.-M. Benoliel, P. Bongrand, A. Ryter, and G. I. Bell. 1982. Concanavalin-A-mediated thymocyte agglutination: a model for a quantitative study of cell adhesion. *J. Cell. Sci.* 56:21–48.

Chien, S., and K.-M. Jan. 1973. Ultrastructural basis of the mechanism of rouleaux formation. *Microvasc. Res.* 5:155–166.

Chien, S., L. A. Sung, S. Kim, A. M. Burke, and S. Usami. 1977. Determination of aggregation force in rouleaux by fluid mechanical technique. *Microvasc. Res.* 13:327–333.

Chien, S., L. A. Sung, S. Simchon, M. M. L. Lee, K.-M. Jan, and R. Skalak. 1983. Energy balance in red cell interactions. *Ann. NY Acad. Sci.* 416:190–206.

Curtis, A. S. G. 1969. The measurement of cell adhesiveness by an absolute method. *J. Embryol. Exp. Morphol.* 22:305–325.

Curtis, A. S. G. 1970. On the occurrence of specific adhesion between cells. *J. Embryol. Exp. Morphol.* 23:253–272.

Curtis, A. S. G., and L. M. Hocking. 1970. Collission efficiency of equal spherical particles in a shear flow. *Trans. Faraday Soc.* 66:1381–1390.

Economidou, J., N. C. Hughes-Jones, and B. Gardner. 1967. Quantitative measurements concerning A and B antigen sites. *Vox Sang.* 12:321–328.

Evans, E. A. 1980. Minimum energy analysis of membrane deformation applied to pipet aspiration and surface adhesion of red blood cells. *Biophys. J.* 30:265–284.

Evans, E. A., and K. Buxbaum. 1981. Affinity of red blood cell membrane for particle surfaces measured by the extent of particle encapsulation. *Biophys. J.* 34:1–12.

Evans, E. A., and A. Leung. 1984. Adhesivity and rigidity of red blood cell membrane in relation to WGA binding. *J. Cell Biol.* 98:1201–1208.

Evans, E. A., and V. A. Parsegina. 1983. Energetics of membrane deformation and adhesion in cell and vesicle aggregation. *Ann. NY Acad. Sci.* 41:13–33.

Feinstein, A., and E. A. Munn. 1969. Conformation of the free and antigen-bound IgM antibody molecules. *Nature (Lond.).* 224:1307–1309.

Goldsmith, H. L., and S. G. Mason. 1967. The microrheology of dispersions. *In* Rheology: Theory and Applications. Vol. 4. F. R. Eirich, editor. Academic Press, Inc., New York. 85–250.

Jeffery, G. B. 1922. On the motion of ellipsoidal particles immersed in a viscous fluid. *Proc. R. Soc. Lond. A.* 102:161–179.

Lerche, D. 1983. The role of electrostatic and structural properties of the cell surface in the energetics of cell-cell and cell-surface interaction. *Ann. NY Acad. Sci.* 416:66–81.

Levine, S., M. Levine, K. A. Sharp, and D. E. Brooks. 1983. Theory of electrokinetic behavior of human erythrocytes. *Biophys. J.* 42:127–135.

Lin, C. J., K. J. Lee, and N. F. Sather. 1970. Slow motion of two spheres in a shear field. *J. Fluid Mech.* 43:35–47.

Parsegian, V. A., and D. Gingell. 1972. Forces between biological cell membranes. *J. Adhesion.* 4:299–306.

Schmid-Schönbein, H., E. Volger, and H. J. Klose. 1972. Microrheology and light transmission of blood. II. The photometric quantification of red cell aggregate formation and dispersion in flow. *Pflügers Arch.* 333:140–155.

Takamura, K., H. L. Goldsmith, and S. G. Mason. 1979. The microrheology of colloidal dispersions. IX. Effects of simple and polyelectrolytes on rotation of doublets of spheres. *J. Colloid Interface Sci.* 72:385–400.

Takamura, K., H. L. Goldsmith, and S. G. Mason. 1981*a*. The microrheology of colloidal dispersions. XII. Trajectories of orthokinetic pair-collisions of latex spheres in a simple electrolyte. *J. Colloid Interface Sci.* 82:175–189.

Takamura, K., H. L. Goldsmith, and S. G. Mason. 1981*b*. The microrheology of colloidal dispersions. XIII. Trajectories of orthokinetic pair-collisions of latex spheres in a cationic polyelectrolyte. *J. Colloid Interface Sci.* 82:190–202.

Tha, S. P., J. Shuster, and H. L. Goldsmith. 1986. Interaction forces between red cells agglutinated by antibody. II. Measurement of hydrodynamic force of break-up. *Biophys. J.* 50:2017–2026.

Van de Ven, T. G. M., and S. G. Mason. 1976*a*. The microrheology of colloidal dispersions. IV. Pairs of interacting spheres in shear flow. *J. Colloid Interface Sci.* 57:505–516.

Van de Ven, T. G. M., and S. G. Mason. 1976*b*. The microrheology of colloidal dispersions. V. Primary and secondary doublets of spheres in shear flow. *J. Colloid Interface Sci.* 57:517–534.

Van de Ven, T. G. M., and S. G. Mason. 1977. The microrheology of colloidal dispersions. VII. Orthokinetic doublet formation of spheres. *Colloid Polymer Sci.* 255:468–479.

Van Oss, C. J., and D. R. Absolom. 1983. Zeta potentials, van der Waals forces and hemagglutination. *Vox Sang.* 44:183–190.