THE EQUILIBRIUM SIZE DISTRIBUTION OF ROULEAUX

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ABSTRACT Rouleaux are formed by the aggregation of red blood cells in the presence of macromolecules that bridge the membranes of adherent erythrocytes. We compute the size and degree of branching of rouleaux for macroscopic systems in thermal equilibrium in the absence of fluid flow. Using techniques from statistical mechanics, analytical expressions are derived for (*a*) the average number of rouleaux consisting of *n* cells and having *m* branch points; (*b*) the average number of cells per rouleau; (*c*) the average number of branch points per rouleau; and (*d*) the number of rouleaux with *n* cells, $n = 1, 2, \ldots$, in a system containing a total of *N* cells. We also present the results of numerical evaluations to establish the validity of asymptotic expressions that simplify our formal analytic results.

I. INTRODUCTION

Under normal physiological conditions, red blood cells tend to aggregate into long, cylindrical, and sometimes branched objects called "rouleaux" (Fig. 1). Rouleau formation is of general interest as a model system for the study of cellular adhesion and aggregation, and is at the same time of physiological significance in the hemodynamics of the microcirculation. Erythrocyte aggregation is also of clinical interest since the appearance of large rouleaux is used diagnostically as an indicator of elevated blood plasma concentrations of certain macromolecules such as immunoglobulin and fibrinogen (cf. Reich, 1978).

Samsel and Perelson (1982 and manuscript in preparation[1]) present a detailed study of the kinetics of rouleau formation. The equilibrium distribution of rouleaux, which should correspond to the infinite time limit of the kinetic theory of Samsel and Perelson, can be calculated analytically in a relatively simple way using the techniques of statistical mechanics. The presentation of the method and the resulting equilibrium size distribution calculated under simplifying assumptions is the subject of this paper. A future paper of ours[2] will discuss mathematical derivations and size distributions calculated to somewhat greater accuracy using more complex assumptions.

In the analysis presented here, we only consider erythrocyte aggregation in the absence of fluid flow. Hydrodynamic aspects of rouleau formation as studied, for example, by Adler (1979) will not be dealt with. Qualitatively, one expects the following situation: if a rouleau is immersed in a viscous fluid (blood plasma) and subject to a shear field with a velocity gradient *G*, the movement of the rouleau will be relatively simple to describe if the rouleau is large. A large rouleau will be highly branched and can, therefore, be described approximately by a porous sphere. The movement of the rouleau is a superposition of a uniform translation of its center and a rotation with a constant angular velocity $\omega = \frac{1}{2} G$. Problems of this type have recently been discussed in detail (Wiegel, 1980). The fluid will permeate the rouleau to a certain extent and the frictional forces that the fluid exerts on different parts of the rouleau can tear the rouleau apart. A short rouleau, on the other hand, will have the shape of a single short cylinder. In a shear flow field the fluid exerts a torque on the surface of the rouleau which causes it to rotate (Goldsmith, 1966 and 1968). In addition, both compressive and tensile forces act on the rouleau while it is rotating causing it to bend (Goldsmith, 1966 and 1968) and possibly break. Hence it can be expected that the rouleau size distribution under shear will be shifted towards smaller sizes as compared with the distribution in a fluid at rest.

In this paper the following problem is considered: *N* red blood cells are placed in a fluid at rest in a volume *V*, at an absolute temperature *T*. As these cells form rouleaux, two cells can adhere with a binding energy $-E_A < 0$ (Fig. 2 *a*), or three cells can form a branch point, which has a binding energy $-E_B < 0$ (Fig. 2 *b*). As a result of single cells or aggregates adding to existing aggregates, the red cells form treelike structures (see Fig. 1). Samsel and Perelson (1982) discuss in detail the various mechanisms by which branched rouleaux can form. This process of tree formation is counteracted by thermal motion (Brownian movement) which tends to break up rouleaux, especially large ones which can disintegrate in many different ways. Our

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[1] Samsel, R. W., and A. S. Perelson. Manuscript in preparation.

[2] Wiegel, F. W., and A. S. Perelson. The statistical mechanics of red cell aggregation: the distribution of rouleaux in thermal equilibrium. Manuscript submitted for publication.

FIGURE 1 Typical rouleaux obtained from fresh human blood, anticoagulated with EDTA, centrifuged and resuspended in its own plasma at a hematocrit of 1%.

goal is to calculate the resulting equilibrium size distribution of rouleaux, i.e., the number of rouleaux of a given size after an infinitely long time. We also calculate the equilibrium distribution of the number of branch points in rouleaux of a given size.

In the following sections the Boltzmann factors corresponding to the binding energies for chain elongation and chain branching will be denoted by

$$\alpha = \exp(E_A/k_BT)$$

and

$$\beta = \exp(E_B/k_BT), \tag{1.1}$$

respectively, where k_B is Boltzmann's constant. The values of E_A and E_B depend of course on the type and concentration of the macromolecules that form the intercellular bridges mediating cellular adhesion, the ionic composition of the medium, and the viscoelastic properties of the red cell membrane (cf. Chien, 1980 and 1981). We expect

$$E_A \leqq E_B \leqq 2E_A. \tag{1.2}$$

If E_A were greater than E_B then no branching would be present at equilibrium, contrary to observation. The three cells forming a branch point can at best adhere over an area which is twice that over which two cells can adhere (see Fig. 2) and consequently we expect $E_B \leqq 2E_A$. For stable adhesion both E_A and E_B will be large compared with the thermal energy k_BT, and hence α and β will be large when compared with unity.

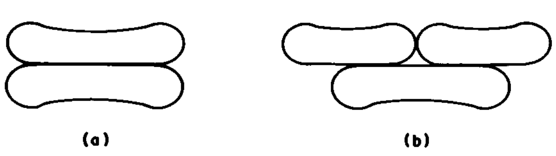

FIGURE 2 *a* Two cells adhere with energy $-E_A < 0$. (*b*) Three cells adhere forming a branch point with energy $-E_B < 0$.

Treelike structures, which play a central role in our analysis, have been studied extensively in the theory of graphs (cf. Harary, 1969). They have found many applications in statistical mechanics; the most closely related to the present work has been in the theory of helix-coil transitions in synthetic self-complementary polynucleotides (Gō, 1967; Hijmans, 1967; De Gennes, 1968; Wiegel, 1982). At very large rouleau sizes, the branching tree approximation may break down as a result of the formation of cycles in which a branch of a tree will attach itself to some other branch of the same tree.

The following quantities can be used to characterize the rouleau size distribution at equilibrium: (*a*) the average number of rouleaux which consist of *n* cells and have *m* branch points, $R^*_{n,m}$. This quantity leads, among others, to the degree of branching of the "average" rouleau and should, therefore, play a role in determining the sedimentation rate of a rouleau; (*b*) the average number of rouleaux consisting of *n* cells, R^*_n; (*c*) the average number of cells per rouleau $\langle n \rangle$; and (*d*) the average number of branch points per rouleau, $\langle m \rangle$.

II. GENERAL CONSIDERATIONS

The general scheme of equilibrium statistical mechanics can be applied to the computation of the rouleau size distribution. For a review of the methods we use, we refer the reader to the existing monographs on the subject (for example, Pathria, 1972; Huang, 1963).

A "macrostate" of the system is a collection of numbers $\{R_{n,m}\} \equiv (R_{1,0}, R_{2,0}, R_{3,0}, \ldots)$, where $R_{n,m}$ denotes the number of rouleaux consisting of exactly *n* cells and having *m* branch points. A "microstate" of the system is a particular assignment of the *N* red blood cells to the various rouleaux, and an assignment of the positions of the various rouleaux within the volume *V*.

We first calculate the configuration sum $Q\{R_{n,m}\}$, defined by

$$Q\{R_{n,m}\} = \sum_C{}' \exp[-E(C)/k_BT], \tag{2.1}$$

where the prime indicates a summation over all those microstates that are compatible with the macrostate $\{R_{n,m}\}$ and where $E(C)$ denotes the energy of microstate *C*. The system can be in any of a large number of macrostates. The probability of finding the system in a particular macrostate, $\{R_{n,m}\}$, is simply the ratio of $Q\{R_{n,m}\}$ to $\Sigma Q\{R_{n,m}\}$, where the sum is taken over all possible macrostates for the system. Having determined $Q\{R_{n,m}\}$ we next calculate the equilibrium distribution, $\{R^*_{n,m}\}$, by the standard technique of maximizing $Q\{R_{n,m}\}$ with respect to variations of the $R_{n,m}$ that are compatible with the constraint that the total number of cells is constant

$$\sum_{n=1}^{\infty} nR_n = N, \tag{2.2}$$

where R_n denotes the number of rouleaux containing exactly n cells, i.e.,

$$R_n = \sum_{m=0}^{\infty} R_{n,m}. \tag{2.3}$$

This method of calculation tacitly assumes that we can identify the equilibrium distribution, $\{R^*_{n,m}\}$, with the most probable distribution found by maximizing $Q\{R_{n,m}\}$. As discussed in statistical mechanics texts (cf. Reif, 1965) this assumption is only valid when the number of states is so large that fluctuations around the most probable distribution are neglible. One can rigorously show that this is the case in the "thermodynamic limit," i.e. when $N \longrightarrow \infty$, $V \longrightarrow \infty$, but the cell density N/V remains finite. Thus, our method will only be valid for studies employing a large number of cells and a large volume of blood, as is usually the case. Using the techniques developed by Donoghue and Gibbs (1979) it should be possible to extend our work to systems containing small numbers of red cells.

The calculation of $Q\{R_{n,m}\}$ proceeds in several steps. First consider a rouleau with n cells and m branch points in which (*a*) the n cells are assumed to be indistinguishable and (*b*) the rouleau is assumed to be at some fixed position in space. Assumptions (*a*) and (*b*) will be relaxed in later steps of the calculation. Such a rouleau can be in any of a large number of microstates. Weighting each such microstate by its probability of occurrence via a Boltzmann factor, we are led to define the configuration sum

$$Q_{n,m} = \sum_{C'} \exp[-E(C')/k_B T] \tag{2.4}$$

of a rouleau containing m branch points and n indistinguishable cells which is fixed in space. The summation in Eq. 2.4 is over all possible configurations (or microstates) C' of such a rouleau. Because the rouleaux we are studying have no loops, i.e., look like trees, one can use standard combinatorial procedures such as generating function methods to count the number of trees with n branches (cells) and m branch points. To fix the rouleau in space we fix the position of one cell, called the root of the tree, and then count the number of "rooted" trees. As we show elsewhere,[2] such a procedure leads to

$$Q_{n,m} = \frac{\beta^m \alpha^{n-2m-1} \binom{n-1}{2m} \binom{2m}{m}}{m+1}. \tag{2.5}$$

Alternatively, one might only fix the position of the center of mass of the rouleau and allow rotations about that position and various internal motions. Under such circumstances there is no distinguished cell in the rouleau and one must count unrooted trees. A slightly different formula for $Q_{n,m}$ then results. Further, one may wish to take into account the fact that a red cell has two faces and add a combinatorial factor to Eq. 2.5. For the purposes of this paper the simple form of Eq. 2.5 suffices. Wiegel and Perelson[2] discuss the alternative forms of $Q_{n,m}$ obtained under these different circumstances.

Red cells are classical as opposed to quantum mechanical particles and hence must be considered distinguishable. Each microstate of a rouleau containing n indistinguishable particles generates $n!$ microstates of a rouleau with n distinguishable particles. Thus microstates arise out of each other by permuting the n cells in the rouleau.

The next step in the calculation is to relax assumption (*b*). The cell representing the position of the rouleau i.e., its root, can be in any of

$$N_0 \equiv V/v \tag{2.6}$$

positions in the volume V, where v is the volume of a single red cell. Thus for each rouleau with n distinguishable cells and m branch points the sum of Boltzmann factors is $N_0 n! Q_{n,m}$.

Now we must take into consideration the fact that there is more than one rouleau present. There are $R_{n,m}$ rouleaux with n cells and m branch points, each of which can be in any of the microstates considered above. Thus $(N_0 n! Q_{n,m})^{R_{n,m}}$ equals the Boltzmann weighted number of states for the $R_{n,m}$ rouleaux with n cells and m branch points. Taking the product of these factors for all values of m and n leads to

$$\prod_{n=1}^{\infty} \prod_{m=0}^{\infty} (N_0 n! Q_{n,m})^{R_{n,m}}, \tag{2.7}$$

the Boltzmann weighted number of states for all the rouleaux corresponding to the macrostate $\{R_{n,m}\}$.

The last step in our computation of $Q\{R_{n,m}\}$ is to determine the number of ways N distinguishable cells can be partitioned into R_1 groups of one cell, R_2 groups of two cells, etc. which correspond to the different rouleaux. The number of ways this partition can be done, $\Omega\{R_n\}$, is given by (Mayer and Mayer, 1940).

$$\Omega\{R_n\} = \frac{N!}{\prod_{n=1}^{\infty} [R_n!(n!)^{R_n}]}. \tag{2.8}$$

For one such partition Eq. 2.7 gives the sum of Boltzmann factors. Hence the total configuration sum

$$Q\{R_{n,m}\} = \Omega\{R_n\} \prod_{n=1}^{\infty} \prod_{m=0}^{\infty} (N_0 n! Q_{n,m})^{R_{n,m}} \tag{2.9}$$

where $\Omega\{R_n\}$ and $Q_{n,m}$ are given by Eqs. 2.8 and 2.5, respectively.

The equilibrium distribution $\{R^*_{n,m}\}$ is found by maximizing $Q\{R_{n,m}\}$, subject to the constraint of Eq. 2.2. As the logarithm is a monotonically increasing function, one can maximize $\ln Q\{R_{n,m}\}$ as well. Using the method of Lagrange multipliers and Stirling's asymptotic formula immediately leads to the equilibrium distribution of rouleaux[2]

$$\frac{R^*_{n,m}}{N_0} = Q_{n,m}\exp(-\lambda n). \tag{2.10}$$

The value of the Lagrange multiplier λ is determined by substituting the last equation into the constraint equation 2.2, yielding the implicit formula

$$\frac{N}{N_0} = \sum_{n=1}^{\infty}\sum_{m=0}^{\infty} nQ_{n,m}\exp(-\lambda n). \tag{2.11}$$

Note that N_0 is proportional to the volume V. Hence $R^*_{n,m}/N_0$, as well as λ, does not depend on N and V separately, but only on the cell density N/V.

To perform a variety of computations it is useful to introduce the "generating function"

$$Q(z,\xi) = \sum_{n=1}^{\infty}\sum_{m=0}^{\infty} Q_{n,m} z^n \xi^m, \tag{2.12}$$

where z and ξ are dummy variables. The substitution of Eq. 2.5 into 2.12 leads[2] to

$$Q(z,\xi) = [2\beta\xi Q_0(z)]^{-1}[1 - \sqrt{1 - 4\beta\xi Q_0^2(z)}]. \tag{2.13}$$

where

$$Q_0(z) = z(1 - \alpha z)^{-1}. \tag{2.14}$$

To see why generating functions are useful, notice that Eq. 2.11, which determines λ, is equivalent to the following equation

$$\frac{N}{N_0} = \left(z\frac{\partial Q}{\partial z}\right)_{z=\exp(-\lambda),\,\xi=1} \tag{2.15}$$

where the expression on the right is evaluated at $z = \exp(-\lambda)$, $\xi = 1$ after the differentiation is performed. Evaluating the derivative in Eq. 2.15 through the use of the chain rule results in the following implicit equation for λ:

$$\frac{N}{N_0} = \frac{1 - \alpha z - [(1 - \alpha z)^2 - 4\beta z^2]^{1/2}}{2\beta z[(1 - \alpha z)^2 - 4\beta z^2]^{1/2}}, \tag{2.16}$$

where $z = \exp(-\lambda)$. As we show in the next section this equation can easily be solved by numerical methods.

It is straightforward to calculate other quantities of interest. For example, using Eqs. 2.10 and 2.12 the average number of cells per rouleau can be expressed in terms of the generating function $Q(z,\xi)$

$$\langle n\rangle \equiv \frac{\sum\limits_{n,m} nR^*_{n,m}}{\sum\limits_{n,m} R^*_{n,m}} = \left(\frac{z}{Q}\frac{\partial Q}{\partial z}\right)_{z=\exp(-\lambda),\,\xi=1}. \tag{2.17}$$

The substitution of Eq. 2.15 into 2.17 results in

$$\langle n\rangle = \frac{N}{N_0 Q[\exp(-\lambda), 1]}, \tag{2.18}$$

which shows that the average number of cells per rouleau grows in proportion to the total number of cells, N. Alternatively, the substitution of Eqs. 2.13, 2.14 and 1.1 into Eq. 2.18 yields a form which illustrates the dependence of $\langle n\rangle$ on the energies E_A and E_B,

$$\langle n\rangle = \{[1 - \exp(E_A/k_BT - \lambda)]^2 - 4\exp(E_B/k_BT - 2\lambda)\}^{-1/2}. \tag{2.19}$$

For the calculation of other statistical properties of rouleaux one can make use of the following relations that, for any rouleau, connect the number (S) of straight segments, the number (I) of internal straight segments and the number (E) of external straight segments with the number (m) of branch points

$$S = 2m + 1, \tag{2.20a}$$

$$I = m - 1, \tag{2.20b}$$

$$E = m + 2, \tag{2.20c}$$

where $m = 0, 1, 2, \ldots$. It is, therefore, only necessary to calculate the average number $\langle m\rangle$ of branch points per rouleau. Using Eqs. 2.10 and 2.12 one finds

$$\langle m\rangle \equiv \frac{\sum\limits_{n,m} mR^*_{n,m}}{\sum\limits_{n,m} R^*_{n,m}} = \left(\frac{\xi}{Q}\frac{\partial Q}{\partial \xi}\right)_{z=\exp(-\lambda),\,\xi=1}. \tag{2.21}$$

from which explicit values of $\langle m\rangle$ can be calculated.

III. NUMERICAL EVALUATIONS AND RESULTS

Our analysis in Section II gave rise to an explicit expression for the configuration sum

$$Q_{n,m} = \frac{\beta^m\alpha^{n-2m-1}\binom{n-1}{2m}\binom{2m}{m}}{m+1}, \tag{3.1}$$

in terms of the energy of adhesion between two cells E_A, $\alpha = \exp(E_A/k_BT)$, and the energy of adhesion among the cells which form a branch E_B, $\beta = \exp(E_B/k_BT)$. The quantity $Q_{n,m}$ determines $R^*_{n,m}$, the equilibrium number of rouleaux with n cells and m branch points, through the equation

$$R^*_{n,m} = N_0 Q_{n,m}\exp(-\lambda n), \tag{3.2}$$

once the Lagrange multiplier λ is found. Making use of the generating function $Q(z,\xi)$ we showed that λ obeys the equation

$$\frac{N}{N_0} = \frac{1 - \alpha \exp(-\lambda) - \{[1 - \alpha \exp(-\lambda)]^2 - 4\beta \exp(-2\lambda)\}^{1/2}}{2\beta \exp(-\lambda)\{[1 - \alpha \exp(-\lambda)]^2 - 4\beta \exp(-2\lambda)\}^{1/2}}. \tag{3.3}$$

The equilibrium number of rouleaux with n cells, R_n^*, can be determined by summation

$$R_n^* = \sum_{m=0}^{\left[\frac{n-1}{2}\right]} R_{n,m}^*, \tag{3.4}$$

where $[(n - 1)/2]$ denotes the greatest integer contained in $(n - 1)/2$. The upper limit in this summation is determined by the fact that $Q_{n,m} = 0$ when $2m > n - 1$. To perform the summation analytically one needs to explicitly sum $Q_{n,m}$ over m to obtain Q_n. Being unable to perform this summation, we have derived[2] an asymptotic analytical formula for Q_n, which is valid for $n \gg 1$. Using this approximate formula for Q_n one can then show[2] that for $n \gg 1$

$$R_n^* \simeq cN_0 n^{-3/2} \mu^n. \tag{3.5}$$

where $c = (2\pi\beta)^{-1/2}(1 + \alpha/2\sqrt{\beta})^{1/2}$ and $\mu = (\alpha + 2\sqrt{\beta}) \exp(-\lambda)$.

Before evaluating Eqs. 3.1–3.5 numerically we need to assign values to the parameters in our theory: N, N_0, V, E_A and E_B. The quantity N/N_0 can be physically interpreted. Since $N_0 \equiv V/v$, where v is the volume of a red cell and V is the total system volume, $N/N_0 = Nv/V$ is the ratio of the volume occupied by red cells to the total volume. This quantity expressed as a percentage is known as the hematocrit. Normal human blood has an hematocrit of ~45% and varies somewhat with sex and age (Miale, 1977). Rouleau formation experiments are performed at this hematocrit and at lower hematocrits, typically 0.5%–5%, where individual red cells and rouleau can be distinguished (cf. Kernick et al., 1973; Pittz et al., 1977). A 1% hematocrit corresponds to 1.07×10^5 erythrocytes/mm^3 (Miale, 1977); thus $N_0/V \simeq 10^7$ red cells/mm^3 and N/V is determined by the experimental hematocrit. In what follows we shall assume that N_0 and N are given per unit volume. Therefore $R_{n,m}^*$ and R_n^* will also be expressed as rouleaux per unit volume.

The values of E_A and E_B are harder to determine. As argued in section I, we can assume $E_A \leqq E_B \leqq 2E_A$. The value of E_A will depend upon the concentration of the bridging macromolecules, the number of bonds made with the cell surface per bridging molecule, the interaction energy per bond, the ionic strength of the solution as well as factors intrinsic to the red cell, including its surface charge, and the elastic properties of the membrane (see Chien, 1981, for a review of these factors). The net aggregation energy (E_A) is thus composed of a number of factors:

$$E_A = E_b - E_e - E_m - E_s, \tag{3.6}$$

an adhesive energy due to macromolecular bridging (E_b), which is counterbalanced by energies due to electrostatic repulsion of negatively charged red cells (E_e), the strain energy of the red cell membrane resisting deformation during aggregation (E_m), and the energy of any applied shear stress (E_s) tending to disaggregate the cells (Chien, 1981). In the absence of fluid flow, the conditions imposed in our analysis, $E_s = 0$. For aggregation due to Dextran 70, with average molecular weight 75,000, Chien (1981) estimates that the aggregation energy per unit area is of order 10^{-4} ergs/cm^2. Since the area of contact between two cells in a rouleau is of order 50 μm^2 (Chien, 1981), at 37°C the net adhesion energy E_A is of order 10^3 k_BT. The Boltzmann factor α corresponding to this value of E_A is of order 10^{434} and thus too large to fit in most modern computers. Consequently, to numerically evaluate the formulas in our theory and to test our approximate solutions we have chosen to use $E_A = 10$ k_BT, a value significantly smaller than estimated by Chien, but one that could be attained experimentally at sufficiently low concentrations of the bridging macromolecule or by affecting any of the other factors in Eq. 3.6.

There is an alternative justification for using this small value for E_A. If red cells are colliding not by random thermal motion, but by other more vigorous mixing processes, then one can heuristically model such interactions as being due to an effective temperature, which is much greater than 37°C. Kerner (1972), for example, in using statistical mechanical ideas to model animal populations defined an eco-temperature in a somewhat similar spirit. In this view k_BT, rather than defining the thermal energy, is a parameter describing the energetics of random collisions. Unfortunately, it is not clear to what extent this notion can be made precise. Given a set of red cells moving under prescribed fluid dynamical conditions, the collisions that occur probably will not be totally random. Thus one may not be able to rigorously compute an effective temperature on an a priori basis. However, for cells undergoing pseudorandom collisions at a frequency higher than that due to Brownian motion something akin to an effective temperature should be a useful parameter.

For the parameter values within the ranges discussed above we have solved Eqs. 3.1–3.5 on a Los Alamos National Laboratory CDC 7600 computer (Control Data Corp., Minneapolis, Minn.). Using a 5% hematocrit for illustrative purposes, we list in Table I the values of $R_{n,m}^*$ for various values of n and m, computed using a recursion scheme based upon Eqs. 3.1 and 3.2 assuming $E_A = 10$ k_BT and $E_B = 12$ k_BT. For these values of E_A and E_B the rouleaux tend not to be highly branched. When $n < 80$, straight chain rouleaux are present in higher concentrations than branched rouleaux. However, even when $n < 80$,

TABLE I
VALUES OF $R^*_{n,m}$ FOR VARIOUS VALUES OF n AND m*

n	m							
	0	1	2	3	4	5	10	20
1	4.3×10^5							
2	4.2×10^5							
3	4.0×10^5	1.3×10^2						
4	3.8×10^5	3.8×10^2						
5	3.6×10^5	7.3×10^2	8.2×10^{-2}					
6	3.5×10^5	1.2×10^3	3.9×10^{-1}					
7	3.3×10^5	1.7×10^3	1.1	6.3×10^{-5}				
8	3.2×10^5	2.2×10^3	2.5	4.2×10^{-4}				
9	3.1×10^5	2.9×10^3	4.8	1.6×10^{-3}	5.4×10^{-8}			
10	2.9×10^5	3.5×10^3	8.3	4.6×10^{-3}	4.7×10^{-7}			
15	2.3×10^5	7.1×10^3	5.3×10^1	$1.3 \times 10^{-}1$	1.2×10^{-4}	4.2×10^{-8}		
20	1.9×10^5	1.1×10^4	1.6×10^2	9.6×10^{-1}	2.5×10^{-3}	3.1×10^{-6}		
40	7.7×10^4	1.9×10^4	1.4×10^3	4.8×10^1	8.4×10^{-1}	8.8×10^{-3}	1.6×10^{-15}	
50	5.0×10^4	2.0×10^4	2.4×10^3	1.3×10^2	4.0	7.3×10^{-2}	4.3×10^{-13}	2.2×10^{-46}
100	5.5×10^3	8.9×10^3	4.6×10^3	1.2×10^3	1.7×10^2	1.5×10^1	7.1×10^{-7}	9.6×10^{-29}
200	6.6×10^1	4.3×10^2	9.4×10^2	9.9×10^2	6.1×10^2	2.5×10^2	2.9×10^{-2}	2.3×10^{-16}

*$E_A = 10\ k_BT$, $E_B = 12\ k_BT$ and $N/N_0 = 0.05$.

singly branched rouleaux can occur with reasonable frequency relative to the straight chain rouleaux. For example, when $n = 40$ singly branched rouleaux occur at 25% of the frequency of the straight chain rouleaux. For larger values of n, say $n = 100$ or 200, doubly, triply and quadruply branched rouleaux also occur at high relative frequencies. The average number of branches, $\langle m \rangle$, computed over the whole population of rouleaux via Eq. 2.21 shows $\langle m \rangle$ is very small ($\langle m \rangle = 0.35$). At the other hematocrits of interest 1% and 45%, $\langle m \rangle = 0.07$ and 3.2, respectively, thus highly branched rouleaux are not numerous at any hematocrit for these values of E_A and E_B. However, as E_B becomes larger, branching becomes more probable and $\langle m \rangle$ increases. In Fig. 3 we show the effect of increasing E_B, with E_A held fixed at 10 k_BT, over the range specified by Eq. 1.2 with the hematocrit fixed at 5%.

Computing $\langle n \rangle$ with Eq. 2.18 shows that at 5% hematocrit with $E_A = 10\ k_BT$ and $E_B = 12\ kT$ the average rouleau size is 39.5. At 1% and 45% hematocrits, the corresponding values are $\langle n \rangle = 15.9$ and 207.9, respectively. Thus the higher the hematocrit the larger the rouleaux. This can be seen more dramatically in Fig. 4, where we plot the concentration of rouleaux of size n, R^*_n, vs. n for various hematocrits.

The average rouleaux size also increases if either the adhesion energy E_A or the branching energy E_B increases. In Fig. 5 we show the effect on $\langle n \rangle$ of increasing E_B over the range $E_B \leq E_A \leq 2E_B$, with E_A held fixed at 10 k_BT.

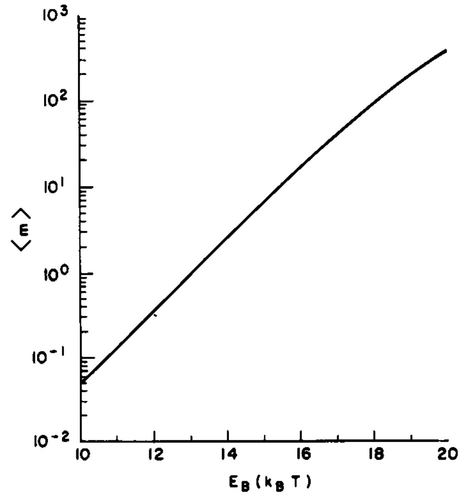

FIGURE 3 The mean number of branch points per rouleau, $\langle m \rangle$, plotted against the branching energy E_B, with $E_A = 10\ k_BT$, $N_0 = 10^7$ cells/mm^3 and $N/N_0 = 5\%$.

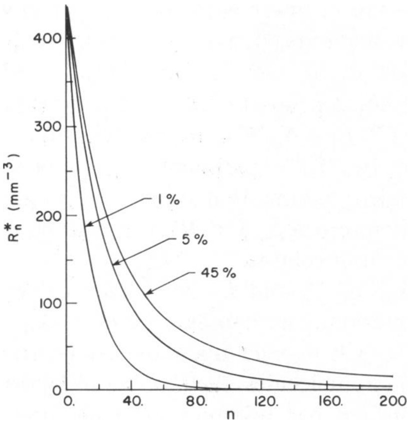

FIGURE 4 The number of rouleaux of size n/mm^3 of blood plotted against n for the hematocrit, N/N_0, equal to 1, 5 and 45%. R^*_n has been expressed in concentration units by choosing $N_0 = 10^7$ cells/mm^3. $E_A = 10\ k_BT$ and $E_B = 12\ k_BT$.

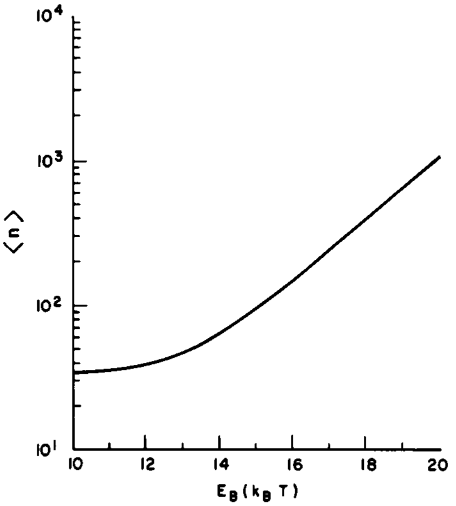

FIGURE 5 The mean number of red cells per rouleau, $\langle n \rangle$, plotted against the branching energy E_B, with $E_A = 10\ k_BT$, $N_0 = 10^7$ cells/mm^3 and $N/N_0 = 5\%$.

Thus as E_B increases, not only are the rouleaux more branched but they are also larger, since each time a branch forms two more cells are added to the rouleau. This qualitative behavior can not easily be predicted from Eq. 2.19 because λ changes if E_B is changed. However, using the asymptotic result given by Eq. 3.5, one can show[2]

$$\langle n \rangle \simeq \frac{N}{2.6\ N_0}\left(\frac{4\pi\beta^{3/2}}{\alpha + 2\sqrt{\beta}}\right)^{1/2}. \qquad (3.7)$$

Thus we see $\langle n \rangle$ increases with β. If we hold α fixed, then $\langle n \rangle$ is proportional to $\beta^{1/2}$ for $2\sqrt{\beta} \gg \alpha$ and a semilogarithmic plot of ln $\langle n \rangle$ vs. E_B should be linear with slope 1/2. This linear dependence is exactly what is seen in Fig. 5 as E_B becomes large.

To test our asymptotic results more carefully we compare in Fig. 6, the exact and asymptotic rouleaux distributions for a 5% hematocrit. Here we see that for small n the asymptotic results are rather poor, but that there is excellent agreement for $n \geq 120$. One can show[2] that the error in the asymptotic formula for R_n^* is due only to the error in the asymptotic formula for Q_n, a quantity whose value is independent of hematocrit. Thus the asymptotic formula for R_n^* at other hematocrits is as accurate as that shown in Fig. 6.

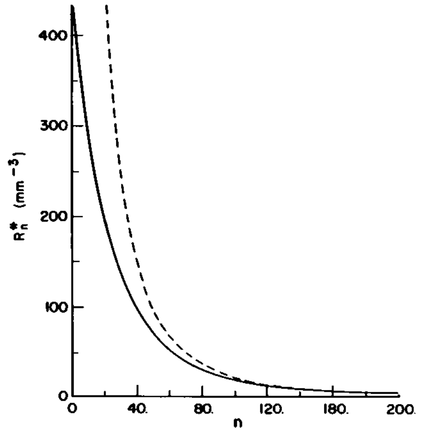

FIGURE 6 The number of rouleaux of size n/mm^3 of blood computed exactly (solid line) and via the asymptotic formula (Eq. 3.5) (dotted line) plotted against n for the hematocrit $N/N_0 = 5\%$. $E_A = 10\ k_BT$ and $E_B = 12\ k_BT$.

IV. DISCUSSION

Using classic methods of equilibrium statistical mechanics we have derived expressions determining the distribution of rouleau sizes and their degree of branching. The system we have considered is highly idealized because we have ignored fluid flow and the sedimentation of erythrocytes, effects which are of importance in vivo and in experimental systems designed to measure rouleau formation. Because erythrocytes are massive, diffusion is a very slow means of mixing red cells. In a vessel at 1 g, red cells will sediment before they undergo large numbers of diffusionally driven collisions. Assuming the cells do not adhere to the bottom of the vessel, rouleaux can continue to form until equilibrium is reached. This is a slow process and one that is difficult to observe. Experimentally the process is usually speeded up by applying a small shear force to an erythrocyte suspension to increase the cell collision rate. If high shear forces are applied, rouleaux are broken up (Usami et al. 1975). Our calculations do not apply to cells under shear and therefore should predict rouleaux that are much larger than are conventionally observed.

The numerical results we reported on in Section III were restricted to very low adhesive energies. At adhesive energies of order $10^3\ k_BT$, the value estimated by Chien (1981), our asymptotic results should hold to a high degree of accuracy. However, from Eq. 3.7 we can surmise that equilibrium will correspond to essentially one large rouleau if the bridging macromolecule is at a sufficient concentration to generate adhesive forces of order $10^3\ k_BT$ and there are no forces other than thermal motion leading to the breakup of rouleaux. Shearing a red cell suspension and observing for short times, as is done in experiment, insures that equilibrium is not established and small discrete rouleaux can be observed. To test our theory low adhesive energies of order $10\ k_BT$ need to be used in which $\langle n \rangle$ remains of order 10^2. For this energy the predictions of our theory are presented in Section III. The nonequilibrium formation of rouleaux observed at short times is dealt with in Samsel and Perelson (1982).

This work was performed under the auspices of the U. S. Department of Energy and supported by NATO research grant RG 067.80. Dr. Perelson is the recipient of an National Institutes of Health Research Career Development Award 7K04 AI 00450-02. This work is dedicated to Aharon Katchalsky-Katzir, a pioneer of the macromolecular bridging hypothesis, whose spirit lives on despite his untimely death at the hands of terrorists.

Received for publication 7 January 1981, and in revised form 4 September 1981.

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