Models for the Specific Adhesion of Cells to Cells

A theoretical framework for adhesion mediated by reversible bonds between cell surface molecules

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Summary. A theoretical framework is proposed for the analysis of adhesion between cells or of cells to surfaces when the adhesion is mediated by reversible bonds between specific molecules such as antigen and antibody, lectin and carbohydrate, or enzyme and substrate. From a knowledge of the reaction rates for reactants in solution and of their diffusion constants both in solution and on membranes, it is possible to estimate reaction rates for membrane-bound reactants. Two models are developed for predicting the rate of bond formation between cells and are compared with experiments. The force required to separate two cells is shown to be greater than the expected electrical forces between cells, and of the same order of magnitude as the forces required to pull gangliosides and perhaps some integral membrane proteins out of the cell membrane.

The social behavior of a cell in a multicellular organism is significantly affected by contacts with other cells or with biological substrata such as collagen. These contacts often involve adhesion of such specificity as to suggest mediation by specific receptor molecules on the cells, and in a few cases there is some definite information about the receptors that are involved.

For example, during aggregation of the slime mold *Dictyostelium discoideum*, cell-to-cell adhesion can be blocked by univalent antibody directed against specific cell surface antigens (*1*). Much larger numbers of univalent antibodies bound to other surface structures do not affect adhesion. Specific aggregation properties of exponentially growing amoebas have been related to differences in glycoprotein composition (*2*). The preferential adhesion between specific cells at certain stages of development of embryos has been studied, and both specific cell surface receptors and diffusible factors have been implicated (*3, 4*). In immunology, lymphocytes can bind to cells that have surface antigens, and collaboration between specific antigen-recognizing and -binding cells is often required for the initiation of immune responses (*5*). Insofar as such collaboration involves cell-to-cell contact, clusters of antibody-bearing cells linked by antigens are probably involved. As a final example, the LETS (large, external transformation sensitive) glycoprotein recently identified on cell surfaces (*6*) appears to bind to specific sites on the collagen of basement membranes (*7*).

In these examples, and perhaps generally, adhesion of a cell to another cell or to a surface may be mediated by specific receptor-receptor or receptor-ligand bonds. At present, mechanisms of cell aggregation and adhesion are under intensive study not only for their intrinsic interest but also because tumor cells differ from their normal counterparts in anchorage dependence of growth, aggregation by lectins, and other properties connected with adhesion (*8*). As cancer cells are by definition invasive, altered adhesiveness may be a fundamental property of the cancer cell (*9*).

Adhesion is difficult to study in vivo except for the simplest of multicellular organisms such as slime molds. Therefore a number of in vitro assays have been developed in an attempt to elucidate the mechanisms of adhesion. Many have examined the attachment of normal or transformed mammalian cells, usually of fibroblasts to surfaces. This now appears to be a rather complicated process, involving the adsorption of certain serum proteins to the surface, followed by attachment of the cell to these proteins at certain sites, followed by active spreading (*10, 11*). Lectins or specific antibodies on the surface can produce attachment (*10*). Another popular assay has involved the agglutination of cells by lectins such as concanavalin A, which can form bridges between carbohydrates on adjacent cells (*12, 13*). Although differences between the agglutination of normal and transformed cells have typically been found, the reasons for these differences have proved elusive (*13, 14*).

Other assays involving adhesion between cells are used in immunology. For example, in rosette assays, red cells may be bound to lymphocytes by means of specific antibodies (*15*). The number of antibodies per red cell and the binding constants can be varied, so the conditions for adhesion are more easily studied in this system than in many others (*16*).

Among the bonds that have been considered as mediating specific adhesion are those between antigen and antibody, lectin and carbohydrate, and enzyme and substrate. Many years ago, Tyler (*17*) and Weiss (*18*) suggested that antigen-antibody bonds between cells determine the specificity in cell-to-cell contact. Although lectin-carbohydrate bonds are generally not considered to be present between cells in multicellular organisms (*19*), it has been suggested that bonds between enzymes on one cell and substrates on another may be common. In particular, glycosyltransferases, which catalyze the addition of sugars to specific polysaccharides, have been found on the membranes of cells (*20, 21*). In the absence of free sugar, such enzymes could bind to the complementary polysaccharides that may be present on glycoproteins of adjacent cells. In this connection, a recent report (*22*) that the immune response (Ia) antigens on B cells are specific oligosaccharides, possibly catalyzed by transferases on T cells, is of interest. However, others have claimed that these antigens are polypeptides (*23*).

Despite great interest in cellular adhesion and a plethora of experimental approaches and speculation, there has been no theoretical framework for quantitatively assessing the role that specific bonds may play in adhesion. The object of this article is to introduce such a theo-

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retical framework. Prior theoretical analyses have considered the electrical forces between cells (or between a cell and a surface), which arise because the interacting bodies are charged and polarizable. These relatively nonspecific forces are briefly reviewed in the next section, after which a model of specific adhesions is developed.

The elements of the theory may be understood by considering an antibody-bearing cell, which may adhere to a cell that has complementary surface antigens. Suppose that the interactions between the individual antibody and antigen molecules in solution are understood, so that forward and reverse rate constants for these elementary bimolecular reactions are known. Suppose also that we know the number of cell surface antibody and antigen molecules on the respective cells and that they are more or less free to move about on the membranes. If the two cells are adjacent, then occasionally an antibody molecule on one cell will encounter an antigen molecule on the other cell and a bond may be established. The first aim of the theory is to deduce rates of bond formation and breakage from the elementary rate constants, the number of receptors per unit cell surface area, and their mobility on the membranes. In addition, the forces that are required to separate two cells that are attached by some number of bonds are estimated and compared with other forces in vitro and in vivo, including the force required to extract a receptor from a cell membrane.

Nonspecific Electrical Forces

The theoretical basis for electrical forces between cells has been reviewed (*24, 25*). In general, cells carry a net negative electrical charge and thus tend to repel each other by the electrostatic force. However, this force is greatly screened by ions in the medium separating the cells; for physiological media, the Debye screening distance is of the order of 1 nanometer. There is additional complexity because most of the charge arises from the dissociation of ionizable groups on the cell surface. For example, on erythrocytes, most of the charge is found on carboxyl groups of sialic acids of the surface glycoproteins (*26*). Since these groups may lie at some considerable but uncertain distances ($\lesssim$ 10 nm) from the lipid bilayer of the plasma membrane (*27*), there is uncertainty in calculating the electrostatic force as a function of distance between bilayers.

In addition, the electrodynamic or van der Waals forces between cells are expected to be attractive and of longer range than the repulsive electrostatic forces. The net result is an energy-separation curve such as that sketched in Fig. 1. This suggests that cells will be attracted to each other until they are separated by a distance equal to the secondary minimum in Fig. 1. In numerical calculations (*24*), secondary minima were estimated for cell separations of $\lesssim$ 7 nm. The corresponding forces needed to separate the cells were estimated to be $\sim 10^{-5}$ dyne per square micrometer of cell area. Numerical values depend on the model chosen for the cell periphery, and the attractive van der Waals energy has been estimated for a wide range of plasma membrane and glycoprotein coat compositions and thicknesses (*25*). For a cell separation of 5.0 nm, the interaction energy was $\sim 2 \times 10^{-11}$ erg/μm^2 = 500 kT/μm^2, where k is the Boltzmann constant and T is absolute temperature.

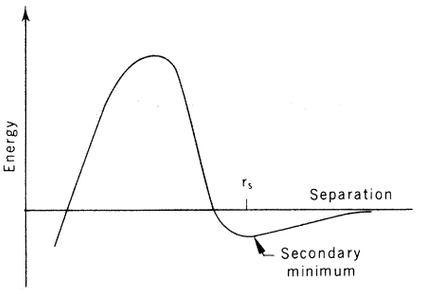


Fig. 1. Energy due to electrostatic and electrodynamic forces plotted against the separation between two planar cell membranes.

Experimental evidence has been obtained for long-range attractive forces between red cells and metallic or hydrocarbon surfaces (*28*). Moreover, measurements have been made of the force as a function of the separation of lecithin bilayers (*29*), and the results are in qualitative agreement with the theory. Thus it appears likely that there is a long-range attractive force of electrodynamic origin between cells or between a cell and a substrate. This attractive force plus the repulsive electrostatic force will favor a separation corresponding to the secondary minimum. Although there is considerable uncertainty in this equilibrium separation distance and the force required to further separate the cells, the model calculations (*24, 25*) indicate that the separation between lipid bilayers may be of the order of 10 nm. Cells separated by such distances should have opportunities for contacts between their glycoproteins and glycolipids and for the formation of specific bonds.

I have characterized the electrical forces as nonspecific because they are expected to be present between all cells. However, the electrodynamic force will lead to a preferential attractive force between like cells in a mixed population (*24, 25*).

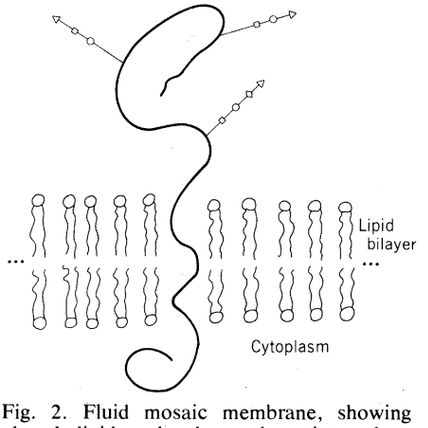


Fig. 2. Fluid mosaic membrane, showing phospholipid molecules and an integral or transmembrane glycoprotein with oligosaccharides in the extracellular region.

Model for the Kinetics of Specific Bond Formation

I assume that the cell periphery is as described by the fluid mosaic model (*27*). In this model, the cell membrane is assumed to consist of a phospholipid bilayer in which various proteins are inserted and retained by virtue of the favorable free energy of their hydrophobic amino acids in the lipid as compared to the aqueous environment. The protein and lipid masses are comparable. Some of the proteins extend all the way through the membrane and can thus interact simultaneously with both cytoplasmic and external molecules; these are called integral proteins. Other proteins, called peripheral, are more loosely attached, possibly to integral proteins. External portions of the proteins are frequently coupled to oligosaccharides, forming glycoproteins. The most common membrane proteins are probably integral glycoproteins (*30*), as sketched in Fig. 2. In addition, some cell surface mucopolysaccharides are probably coupled to integral proteins (*31*).

Membrane proteins are more or less free to move in the plane of the lipid bilayer (*27, 30*), and translational diffusion coefficients have been measured (*32*). The diffusion coefficient depends not only on the type of protein and cell under consideration but also on whether the protein is bound to other molecules in either the cytoplasm or the external medium (*33, 34*). The relationship between translational and rotational diffusion has

also been considered, and they have been shown to be quite different for diffusion in two dimensions as compared to three (*35*).

Consider a particular membrane protein that can form a specific bond with some external molecule. For convenience I call the membrane protein a receptor and the other molecule a ligand, recognizing that the latter may in some cases be itself a protein bound to another cell or surface. For example, the receptor may be an antibody and the ligand an antigen, or the receptor may be an oligosaccharide and the ligand a lectin or glycosyltransferase. Suppose the reaction between the receptor and ligand in solution is well understood and that it can be characterized as a reversible bimolecular reaction. When the receptor is attached to a cell surface, in such a way that its reactive site is accessible, and the ligand is in solution, it is reasonable to suppose as a first approximation that the reaction kinetics are not much changed. However, if both ligand and receptor are attached to cell surfaces, the reaction kinetics will be very different. My first objective is to estimate the reaction rates for surface-bound reactants in terms of the reaction rates for both reactants in solution and their diffusion coefficients in the membrane.

Rate Constants

Consider two cells that are, at least locally, close enough to one another that receptors on one can interact with ligands on the other. When the cells are first brought to this position, any particular receptor is unlikely to have a ligand sufficiently close to be able to form a bond, but as one or both reactants diffuse about in their respective membranes opportunities for bond formation will occur. To estimate the opportunity rate I utilize the notion of the encounter complex (*36*). In this approach the reaction, whether of reactants in solution or on cell surfaces, is conceptually separated into two steps. In the first step the reactants simply encounter each other—that is, they diffuse into sufficiently close proximity to permit the second or reaction step. The reaction may thus be written as

$$A + B \underset{d_-}{\overset{d_+}{\rightleftharpoons}} AB \underset{r_-}{\overset{r_+}{\rightleftharpoons}} C \qquad (1)$$

where d_+ and d_- are the rates of formation and dissolution of the encounter complex, AB, while r_+ and r_- are the forward and reverse rate constants for formation of the bound state, C, from AB. I assume that reactants form an encounter complex whenever they are separated by R_{AB}, the encounter distance.

For reactants in solution the rate constants for the encounter step are (*36*)

$$d_+^s = 4\pi[D_s(A) + D_s(B)]R_{AB} \qquad (2)$$

$$d_-^s = 3[D_s(A) + D_s(B)]R_{AB}^{-2} \qquad (3)$$

where $D_s(A)$ and $D_s(B)$ are the translational diffusion constants of A and B in solution. Note that the equilibrium constant for the encounter step

$$K_d^s = \frac{d_+^s}{d_-^s} = \frac{4\pi}{3} R_{AB}^3 \qquad (4)$$

is simply the volume of a sphere of radius R_{AB}.

However, when both reactants are attached to membranes, either to the same membrane or to two different membranes, separated by a distance $\lesssim R_{AB}$, it can be shown that (*37*)

$$d_+^m = 2\pi[D_m(A) + D_m(B)] \qquad (5)$$

$$d_-^m = 2[D_m(A) + D_m(B)]R_{AB}^{-2} \qquad (6)$$

where $D_m(A)$ and $D_m(B)$ are translational diffusion constants for receptor motion in the membrane. In this case the equilibrium constant for the encounter step is

$$K_d^m = \pi R_{AB}^2 \qquad (7)$$

which is the area of a disk of radius R_{AB}. Note that for membrane-bound reactants, the concentrations are given per unit area rather than per unit volume, as in solution.

Under many conditions the concentration of the encounter complex is small compared to that of the reactants or product, and it is a good approximation (*36*) to set $d[AB]/dt = 0$ in the kinetic equations. The overall reaction A + B $\rightleftharpoons$ C can then be represented in terms of the rate constants

$$k_+ = \frac{d_+ r_+}{d_- + r_+} \qquad (8)$$

and

$$k_- = \frac{d_- r_-}{d_- + r_+} \qquad (9)$$

Note that the overall equilibrium constant, $K = k_+/k_- = K_d r_+/r_-$, is independent of diffusion constants. However, both forward and reverse rate constants will depend on the diffusion constants whenever d_- is not much greater than r_+. If, in particular, $r_+ >> d_-$, so that the encounter complex is much more likely to react than to dissociate, then $k_+ \simeq d_+$. In this case the forward reaction rate is diffusion-limited. The reverse rate constant, $k_- \simeq d_- r_-/r_+$, will then also be proportional to the diffusion constant. Thus if the diffusion constants are small, as for receptors in a viscous membrane, both forward and reverse rate constants will be small.

For any particular receptor-ligand combination, the procedure for estimating rate constants for membrane-bound reactants (k_+^m and k_-^m) is as follows. First, suppose the solution reaction rates (k_+^s and k_-^s) and the solution diffusion constants are known. Then r_+ and r_- can be deduced from Eqs. 2, 3, 8, and 9. If the diffusion constants are also known for receptor and ligand in the membrane, they can be used in Eqs. 5 and 6, together with the previously determined r_+ and r_- to obtain k_+^m and k_-^m.

For example, reaction rates have been determined for many antigen-antibody bonds (*38, 39*) and have been compared with diffusion limits. In these tabulations, forward reaction rates ranged over three orders of magnitude, from 4×10^5 to $6 \times 10^8 M^{-1}$ sec^{-1}. The larger values are close to the diffusion limit, d_+. For example, for a typical ligand of molecular weight 400, $D_s(B) \simeq 5 \times 10^{-6}$ cm^2/sec. For an antibody molecule $D_s(A) \simeq 5 \times 10^{-7}$ cm^2/sec, and for $R_{AB} \simeq 0.75$ nm, $d_+^s \simeq 5 \times 10^{-12}$ cm^3/sec $= 3 \times 10^9 M^{-1}$ sec^{-1}, $d_-^s \simeq 3 \times 10^9$ sec^{-1}, and $K^s \simeq 1 M^{-1}$. However the reactions can proceed only if antibody and hapten have appropriate orientations (*39*). This effect is likely to reduce d_+^s to the range 10^8 to $10^9 M^{-1}$ sec^{-1} (*39*). Thus it appears that from Eq 8, for hapten-antibody reactions, r_+ is likely to be in the range 10^{-3} d_+^s to 1 d_+^s or 10^6 to 10^9 sec^{-1}.

The reverse reaction rates, k_-^s, varied from 3×10^{-5} to 6×10^3 sec^{-1}, and most of this variation must be attributed to variation in the intrinsic reaction rate, r_- (*39*).

Suppose now that the antibody and antigen molecules are attached to cell membranes. The diffusion constant for antibodies on lymphocytes is $D_m(A) \simeq 10^{-10}$ cm^2/sec (*32*), nearly four orders of magnitude lower than the diffusion constant in solution. If we take $D_m(A) + D_m(B) = 10^{-10}$ cm^2/sec and $R_{AB} = 0.75$ nm, then $d_+^m \simeq 6 \times 10^{-10}$ cm^2/sec and $d_-^m \simeq 4 \times 10^4$ sec^{-1}. Note the units of d_+^m, which are appropriate for measuring reactant concentrations in molecules per unit surface area of membrane. Since d_-^m is so much smaller than d_-^s, it is likely that k_+ will be near the diffusion limit. Moreover k_- will generally be considerably smaller than r_-. This is because reactants that have come apart will, in a viscous medium or on a membrane, tend to recombine before diffusing apart.

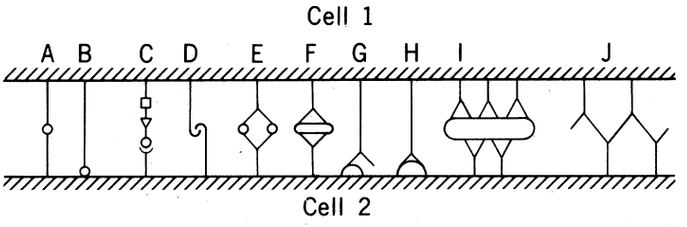


Fig. 3. Some intercellular bonds: (A) two receptors linked by a bivalent ligand, (B) a receptor on cell 1 binding to a ligand on cell 2, (C) an enzyme-substrate bond, (D) a (hypothetical) bond between identical receptors, (E) antibodies (or glycoproteins) bridged by two bivalent antigens (or lectins), (F) two antibodies bridged by a multivalent antigen, (G) antibody binding singly to antigen, (H) antibody binding multiply to antigen, (I) multiple antibodies bridged by a multivalent antigen, and (J) complementary antibody bonds.

My main conclusions from the foregoing are that (i) both forward and reverse rate constants will be reduced for membrane-bound reactants, (ii) the forward rate constant may be fairly close to its diffusion limit, and (iii) the equilibrium constant will not depend on the diffusion constants.

I have ignored the rotation of the reacting molecules or any other motions such as waving to and fro of the receptor molecule on the membrane. In a particular system one might be able to take these motions partially into account (*37, 39*). However, the main conclusions are probably not very sensitive to these refinements. I have also neglected transients, which may arise when the cells are first brought close together. That is, some of the receptors may, by chance, immediately find themselves in opposition so that no diffusion is required to initiate some reactions. Only after some time has elapsed will the reaction rates derived above be reasonably accurate. Such refinements may be effected by modifying this theory, but they are not treated in this article.

Reactions

Some of the kinds of binding reactions that are of interest are sketched in Fig. 3. The reactions could be classified in various ways—for example, by the molecular nature of the reactants such as antibody, lectin, or enzyme. In some cases a diffusible ligand links similar receptors on two cells, while in others bonds between complementary receptors are formed. Finally, in some cases multiple bonds to a single receptor or ligand molecule are involved; in other cases they are not. Any theory that would encompass all these cases would be rather cumbersome. I therefore choose two cases to illustrate the method of theoretical formulation.

The first case is the simplest example, namely two adjacent cells having complementary receptors, mobile in their respective membranes, as shown in Fig. 4. Let N_1 and N_2 be the numbers of receptors per unit area of membrane and N_{1f} and N_{2f} the corresponding numbers of unbound receptors. Then if N_b is the number of bound receptors per unit area that serve to bridge the cells

$$N_i = N_{if} + N_b (i = 1, 2) \qquad (10)$$

Moreover, I assume that bond formation is governed by the kinetic equation

$$\frac{dN_b}{dt} = k_+^m N_{1f} N_{2f} - k_-^m N_b \qquad (11)$$

Using Eq. 10, this can be rewritten as

$$\frac{dN_b}{dt} = k_+^m (N_1 - N_b)(N_2 - N_b) - k_-^m N_b \qquad (12)$$

and solved. The rate of bond formation will be maximum when the cells are first brought together, so that $N_b = 0$ and

$$\left(\frac{dN_b}{dt}\right)_{max} = k_+^m N_1 N_2 \qquad (13)$$

and at long times equilibrium will be approached such that the right-hand side of Eq. 12 is zero and

$$N_b = \frac{1}{2}\left(N_1 + N_2 + \frac{1}{K^m}\right) - \frac{1}{2}\left[\left(N_1 + N_2 + \frac{1}{K^m}\right)^2 - 4N_1 N_2\right]^{1/2} \qquad (14)$$

where $K^m = k_+^m / k_-^m$.

Suppose, for example, that k_+^m has a value equal to one-tenth the diffusion limit for membrane-bound reactants (Eq. 5) and that both receptors have diffusion constants of 10^{-10} cm^2/sec (*32*). Then $k_+^m \simeq 1.3 \times 10^{-10}$ cm^2/sec $= 1.3 \times 10^{-2}$ μm^2/sec, so that if N_1 and N_2 are measured per square micrometer, Eq. 13 gives $dN_b/dt \leq 1.3 \times 10^{-2}\ N_1 N_2\ \mu m^{-2}$ sec^{-1}. If one considers a cell such as a small lymphocyte, with radius $\simeq 4$ μm, area $\simeq 200$ μm^2, and 10^5 receptors, then $N_1 (= N_2) \simeq 500$ and $dN_b/dt \leq 3 \times 10^3$ μm^{-2} sec^{-1}. The B lymphocytes have approximately 10^5 (*5*) antibody molecules on their surfaces as receptors, so this estimate might hold for a B lymphocyte interacting with another cell that carried 10^5 antigenic determinants. For cells both having 10^3 (or 10^7) determinants, the bond formation rate would be ≤ 0.3 (or 3×10^7) μm^{-2} sec^{-1}.

If the cells are adjacent over substantial fractions of their surfaces, it is apparent from the estimates above that large rates of bond formation may result. However, if the cells are in contact only near the tips of a few microvilli the contact area may be quite small (*40*). Typical microvilli have diameters ~ 0.1 μm and hence the contact area of one tip would be $\sim 10^{-2}$ μm^2. In addition, it should be noted that if the cells are adjacent only over localized areas, then receptors from outside the contact areas can diffuse in to increase the local receptor density and to provide further bonds (*13*). The theoretical treatment of such receptor clustering is a separate problem, which will not be developed in this article. An additional complication is that the diffusion constant, D_m, may be smaller for receptors in the contact area than elsewhere on the surface (*41*). However, it is not clear whether such reduced mobility precedes contact or may be a consequence of it.

A somewhat more complicated model, indicated in Fig. 5, is that in which two cells having similar receptors may be bridged by diffusible bivalent ligands. For somewhat more generality I also allow the receptors on a single cell to be cross-linked by the bivalent ligand and allow a competitive monovalent ligand to be present. Thus the model can simulate the several situations listed in Table 1. The configurations of receptor molecules that are of interest are shown in Fig. 5. Just as for the simpler model, I could write kinetic equations for the various kinds of receptor molecules. Reactions involving free hapten or antigen molecules would have solution rate constants, whereas those involving cross-linked or bridged receptors would have membrane-bound rate constants. Such equations will be published elsewhere (*42*).

Complications arise in applying this model to the analysis of experiments—for example, on the agglutination of cells. When "antigen" is first introduced into the medium, it will immediately begin to bind to the cell surfaces and to cross-link receptors. Only when two cells are adjacent to one another will intercellular binding be possible, and if most of the receptors are cross-linked such binding will be inhibited. Hence the

rate or probability of agglutination will depend on details of the experimental design that influence the relative rates of cross-linking, intercellular encounter, and so on. Similar considerations apply to the agglutination of cells by lectins or by enzyme-substrate interactions, inasmuch as the lectins can also cross-link carbohydrates on the same cell and the enzyme can bind to substrate on its own cells. In addition, cross-linking of receptors may reduce receptor mobility over the whole cell surface by modulating the anchorage of the receptors to cytoplasmic proteins or other components (*33, 43*). Moreover, it should be noted that protrusions on a cell may stick to other portions of the same cell surface.

Before applying these models to the interpretation of any experiments, it is appropriate to consider the strength of the bonds and how many are required to hold two cells together or to hold a cell to a surface.

Strength of Specific Bonds

Consider a bond that is formed between two molecules such as an antigen and antibody. Such bonds derive their stablity from a variety of small free energy changes, which may be associated with electrostatic, van der Waals, or hydrogen bond interactions. The bonds are reversible so that no force is needed to separate the molecules; a little patience will suffice. However, when many bonds are linking two cells together, patience will not suffice because the probability for all the receptors to be simultaneously unbound is very small. To separate the cells a force is required that will fairly rapidly rupture each bond.

Table 1. Possible bonding molecules.

Receptor	Ligand	Inhibitor
Antigen	Antibody	Monovalent antibody (Fab fragment) or hapten*
Antibody	Antigen	Hapten
Carbohydrate	Lectin	Monovalent lectin or sugar*

*In this case the inhibitor binds to the ligand and not to the receptor.

What force is required to "rapidly" break a single antigen-antibody bond? If one begins to separate the molecules along some direction of minimum work, then the free energy must vary with separation as sketched in Fig. 6. It must have a minimum at the equilibrium binding position and work must be done to further separate the molecules. As a force is applied to separate the molecules, the free energy minimum will diminish and, for a sufficiently strong force, disappear. Suppose E_0 is the free energy change on binding and r_0 is the range of the minimum, such that for a force $f_0 = E_0/r_0$ the minimum has vanished. Such a force will evidently rapidly rupture the bond. Measuring E_0 in electron volts and r_0 in nanometers

$$f_0 = 1.6 \times 10^{-5} E_0/r_0 \text{ dynes per bond} \quad (15)$$

For a representative antigen-antibody bond, $E_0 \simeq 0.37$ eV (= 8.5 kcal/mole, $K^s \simeq 10^6 M^{-1}$). The dimension of the binding cleft on an antibody is $\simeq 1$ nm (*39*), so it is possible that r_0 might be this large, which would lead to $f_0 \simeq 6 \times 10^{-6}$ dyne per bond. But the individual interactions that in sum produce the binding have ranges of 0.1 to 0.2 nm, so that r_0 is probably less than 1 nm. In what follows I will use the estimate $r_0 = 0.5$ nm. This could probably be made more reliable for particular well-characterized molecules, but it is probably good to within a factor of 2 or so. This value of r_0 together with $E_0 = 0.37$ eV gives $f_0 \simeq 1.2 \times 10^{-5}$ dyne per bond. Note that for covalent bonds $E_0 \simeq 3$ eV and $r_0 \simeq 0.14$ nm, so that for these $f_0 \simeq 3 \times 10^{-4}$ dyne per bond. Hence the weak receptor-ligand bond will break for a much smaller force than will a covalent bond.

In the remainder of this section these considerations are made more quantitative. Suppose one cell is stuck to another or to a surface by many bonds. Each is reversible and is being stressed by a force tending to separate the cells. In the kinetic theory of the strength of solids (*44*), the lifetime of a bond is written

$$\tau = \tau_0 \exp[(E_0 - \gamma f)/kT] \quad (16)$$

where τ_0 is the reciprocal of a natural frequency of oscillation of atoms in solids ($\sim 10^{-13}$ second), E_0 is the bond energy, f is the applied force per bond, and γ is a parameter that must be determined empirically to account for the structure of the solid and its imperfections. Equation 16 has a wide range of validity for representing the variation of bond lifetime with f and T.

If it is postulated that Eq. 16 can also be applied to receptor-ligand bonds, then it is natural to identify $\tau(f = 0)$ with the inverse reverse rate constant $(k_-)^{-1}$ or more properly $(r_-)^{-1}$. Moreover γ must be approximately r_0 in order that $\tau \simeq \tau_0$ when $f = E_0/r_0$. If representative values for antibody-hapten bonds (*39*) are taken to be $\tau(f = 0) \simeq 0.01$ second, $E_0 = 0.37$ eV, and $kT = 0.0268$ eV ($T = 37°C$), then $\tau_0 = 10^{-8}$ second. This may be compared with the period of a harmonic oscillator fitted to a potential as in Fig. 6: $\tau_h \simeq 2\pi r_0 (E_0 m)^{-1/2}$ where m is the mass of the oscillator. If $m = 200$ daltons, corresponding to a typical hapten, and $r_0 = 0.5$ nm, then $\tau_h \simeq 5 \times 10^{-12}$ second, substantially smaller than τ_0. However, even if τ_h were used instead of τ_0 in Eq. 16 and E_0 adjusted to give $\tau(f = 0) = 0.01$ second, the conclusion about the qualitative value of f would be unchanged.

Using the values of τ_0, E_0, γ, and T cited above, I find the values for the bond lifetime given in Table 2. If Eq. 16 is now introduced into the earlier models

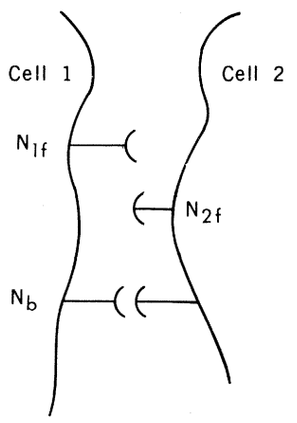
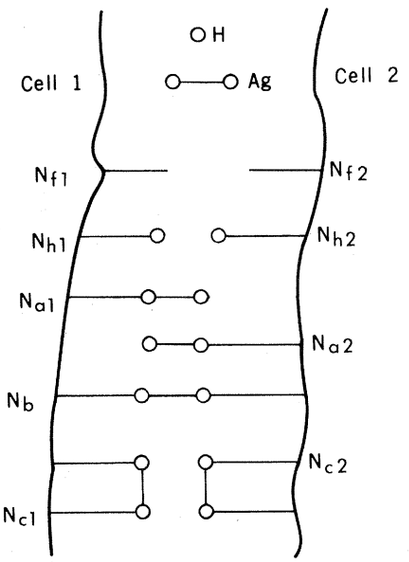


Fig. 4 (left). Simplest model of intercellular bond formation between complementary receptors. Fig. 5 (right). Model of bridge formation by bivalent antigens (*Ag*), in the presence of an inhibiting hapten (*H*). Receptors on either cell can be free (*f*), bound to hapten (*h*), bound to antigen (*a*) and thence to the other cell (*b*), or bound to a receptor on the same cell (*c*).



for bond formation, it is possible to estimate how rapidly any applied force will detach the cells.

Consider two cells that are stuck to each other (or a cell stuck to a surface) by N_b complementary receptors, as in Eq. 12. Suppose that a force F is tending to separate the cells. Assuming that each bond is equally stressed, the force per bond is F/N_b and the reverse rate constant in Eq. 12 should be replaced by

$$k_- \exp(\gamma F/kT\, N_b)$$

If, for simplicity, it is assumed that $N_2 >> N_b$, Eq. 12 is replaced by

$$\frac{dN_b}{dt} = k_+(N_1 - N_b)N_2 - k_-N_b \exp(\gamma F/kT\, N_b) \qquad (17)$$

If the force is zero before $t = 0$, there is initially an equilibrium, $dN_b/dt = 0$, and

$$N_b(0) = KN_2N_1/(1 + KN_2) \qquad (18)$$

where $K = k_+/k_-$. If a small steady force is applied, this will increase the reverse rate constant. After a while, a new equilibrium will be reached with N_b given by Eq. 18, except that K must be multiplied by $\exp(-\gamma F/kTN_b)$. For a large force, however, the second term on the right in Eq. 17 will be much larger than the first, in which case N_b will rapidly go to zero. There will thus exist a critical force, F_c, which is just sufficient to detach the cell. To evaluate F_c, consider the two terms on the right in Eq. 17, which are depicted in Fig. 7 as functions of N_b. Figure 7 shows that for $F = F_c$, the two terms are equal at their point of tangency—that is, when their slopes are equal. Using this fact and setting $\gamma = r_0$, it is straightforward to show that the critical force per potential bond, f_c, is

$$f_c \equiv \frac{F_c}{N_1} = \frac{kT}{r_0}\,\alpha_c \qquad (19)$$

where α_c is the solution of

$$\alpha_c \exp(\alpha_c + 1) = KN_2 \qquad (20)$$

For a large range of KN_2, $\alpha_c \simeq 0.7 \ln(KN_2)$, and using this approximate value together with $r_0 = 0.5$ nm I find that

$$f_c = 8.6 \times 10^{-7}\alpha_c \simeq 6.0 \times 10^{-7}\ln(KN_2) \text{ dynes per bond} \qquad (21)$$

If, for example, $KN_2 = 10^3$, as would be found for $K^s \simeq 10^6 M^{-1}$, $N_2 \simeq 10^3$ bonds per square micrometer, and $R_{AB} \simeq 1$ nm, then Eq. 21 gives $f_c \simeq 4 \times 10^{-6}$ dyne per bond (*45*). This is about a factor of 3 smaller than the earlier estimate of the force f_0 needed to completely eliminate the bond.

Table 2. Times for bond breaking with various forces. Abbreviations: τ, mean time for breaking a single bond, given by Eq. 16 with parameters in the text; t, time to separate cells subject to the force. The critical force is 2.98×10^{-6} dyne per bond.

Force ($\times 10^{-6}$ dyne per bond)	$\log_{10}\tau$ (sec)	$\log_{10} t$ (sec)
0	−2	∞
2	−3	∞
2.98		"∞"
4	−4	−14.7
6	−5	−6
8	−6	−7.1
10	−7	−8.2
12	−8	−9.3

For forces larger than f_c the bonds will break more or less rapidly; numerical solutions of Eq. 17 have been obtained and are shown in Fig. 8. It is apparent that once f is appreciably larger than f_c, the solution has a simple form. It can be found by neglecting the first term on the right in Eq. 17 and solving for the time, t, at which N_b becomes zero. The result is

$$t(F) = \frac{1}{k_-}\,E_1(Y_0) \simeq \frac{\exp(-Y_0)}{k_-(1 + Y_0)} \qquad (22)$$

where $Y_0 = r_0F/kT\, N_b(0) \simeq r_0F/kT\, N_1$ and E_1 is the exponential integral.

These results show that as the force exceeds f_c the cells rapidly tend to separate. Thus, qualitatively at least, the critical force will provide a reasonable estimate of the force required to separate two cells or a cell from a surface. This formalism can be used to estimate critical forces and times for any experimental system, but as a representative value for the critical force I will take in the following discussion the value 4×10^{-6} dyne per bond.

Comparison with Other Forces

The critical force may be compared with other forces to which a cell may be subject. First, I noted that the nonspecific electrical forces between cells have been estimated to be such that an external force of 10^{-5} dyne/μm^2 is required to separate them. The same force would be required to break two or three specific bonds per square micrometer. Inasmuch as the receptor densities on cell surfaces may far exceed these values, it is apparent that specific bonds can cause cells to adhere to each other much more tightly than the nonspecific electrical forces can. An exception might occur if the cells could come close enough that their separation corresponded to the close minimum of Fig. 1. For this to be possible, it may be necessary for the glycoproteins and other nonlipid molecules between the membranes to be removed, which may be an event preceding membrane fusion.

It is also of interest to compare the

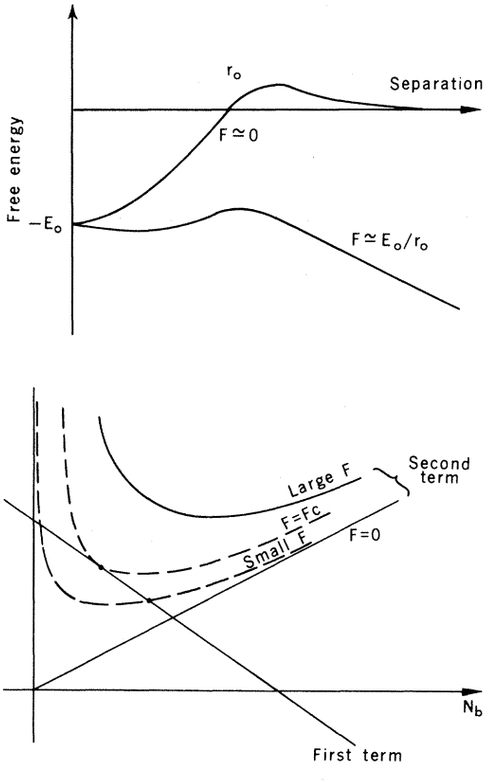


Fig. 6 (top). Free energy plotted against receptor-ligand separation along some direction of minimum work. Zero separation corresponds to the bound state, of free energy $-E_0$ relative to the separated reactants. A force tending to separate the reactants will decrease the free energy linearly with distance, as shown. Fig. 7 (bottom). Equilibrium solutions to Eq. 17 correspond to equality of the first and second terms, shown here. For the critical force, the curves are tangent.

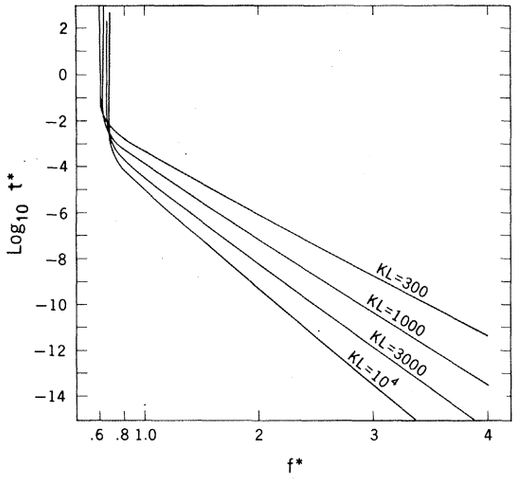


Fig. 8. The time for detachment, $t^* = k_- t$, is shown as a function of the force $f^* = fr_0/kt$ $\ln(KN_2)$. Values of t^* were computed numerically, but Eq. 22 is a good approximation for $f^* \gtrsim 1.0$.

critical force with hydrodynamic forces to which a cell may be subject. In particular, if a stationary cell of radius r is exposed to a fluid stream of velocity v, the force on the cell will be given by Stokes law

$$F = 6\pi\eta r v \qquad (23)$$

where η is the fluid viscosity and it is assumed that the flow is laminar. For water at 37°C, $\eta = 7 \times 10^{-3}$ g/cm-sec. For $r = 4$ μm, $F = 5.3 \times 10^{-5}$ v dynes, where the velocity is in centimeters per second.

I can now estimate the number of bonds needed to hold the cell in a fluid stream of velocity v as

$$N_v \simeq 5.3 \times 10^{-5} v/f_c \simeq 13\, v \qquad (24)$$

Equation 24 tells us, for example, that a cell that is held by 13 bonds should resist fluid velocities of $\lesssim 1$ cm/sec.

In many experimental situations the cell is adhering to a surface or to other cells past which a fluid streams. In such cases the velocity field will be nonuniform and the forces tending to break the bonds may be largely shearing rather than tensile. These complications are not treated in this article. Insofar as the bonding molecules may have some freedom to orient themselves relative to the plane of the membrane, a macroscopic shearing stress across a cell-surface interface may be translated into stresses on the individual bonds that are primarily tensile. However, a uniform stress per bond is unlikely.

In a variety of experiments cells have been specifically attached to nylon fibers that were coated with lectins or antigens (*46–49*). Under appropriate conditions the attached cells can be removed by plucking the fibers. Both inertial and viscous forces act on cells attached to the vibrating string immersed in a liquid medium.

The inertial forces can be computed from the acceleration of the string. A typical vibration frequency is $\simeq$ 1600 cycles per second (*50*) and the maximum displacement is $\simeq$ 1 mm. It follows that the peak acceleration is $\simeq 10^8$ mm/sec^2 and the maximum force on a lymphocyte of mass $\simeq 3 \times 10^{-10}$ g is $\simeq 3 \times 10^{-3}$ dyne. This would be sufficient to break $\sim$ 750 of our standard bonds. However, only the cells on the surfaces of the fiber that are more or less normal to the direction of motion will experience these tensile forces. For most cells on other portions of the fiber, it can be seen that the viscous drag forces are about an order of magnitude larger than the inertial forces. These drag forces will have a large shearing component. Qualitatively, it thus appears that cells having $\lesssim 10^4$ bonds will be mostly removed from the fiber.

For example, a fiber can be completely coated with the lectin concanavalin A, giving $\sim 2 \times 10^4$ lectin molecules per square micrometer (*49*). If a cell could be bound to the fiber by as many as 2×10^4 bonds per square micrometer, and if the contact area were $\sim r^2 = 16$ μm^2, this would give $\lesssim 3 \times 10^5$ bonds per cell. It would appear that such a tightly bound cell could not be removed by plucking the fiber. Yet, in fact, cells have been removed by plucking (*46*), at least for a concanavalin A density of half that cited above. (At the density given above, the cell membranes were often damaged by plucking.)

It thus appears likely that only a small fraction ($\lesssim$ 10 percent) of the maximum possible number of bonds are actually formed when the cell sticks to the fiber. This might be because the actual contact area is substantially less than r^2 or because receptor-ligand bonds cannot form so as to nearly cover the (local) cell surface. Further experiments that are more nearly designed to test the theory should be helpful in testing these alternatives.

It is of some interest to make comparisons with other forces in biology, although I will not attempt to explain their origin. First, the tensile strength of muscle has been reported to be 4 kg/cm^2 = 4×10^{-2} dyne/μm^2 (*51*). This corresponds to 10^4 standard bonds per square micrometer. Second, measurements of the adhesive force between cells (*52*) have yielded values of $\sim$ 1 dyne per cell. This corresponds to $\sim 2.5 \times 10^5$ bonds per cell surface. Finally, mammalian cell surface tensions have been found to be $\gamma \sim 0.5$ dyne/cm, give or take an order of magnitude (*9*). This corresponds to a cell surface pressure $P = 2\gamma/R \sim 10^4/R$ dyne/cm^2, with R in micrometers. For a cell with $R = 4$ μm, $P \sim 2 \times 10^{-5}$ dyne/μm^2. This is equivalent to the force needed to displace about five bonds per square micrometer. Thus we conclude that relatively few bonds would be sufficient to begin to deform the cell. For a microvillus with $R \sim 0.1$ μm, $P \sim 10^5$ dyne/cm^2, equivalent to about two bonds per (0.1 μm)^2, so that a few bonds to an external surface might stabilize a microvillus tip.

Force to Uproot a Receptor

In an earlier section I estimated the force required to break a typical receptor-ligand bond. I noted then that such a force is very unlikely to break a covalent bond. However, the receptor may pull away from the cell. To assess this possibility, the interactions that hold the receptor to the cell must be specified.

Suppose the receptor is an integral membrane glycoprotein. Such a molecule will be positioned in the membrane so as to maximize the exposure of hydrophobic amino acid residues to the membrane lipids and of hydrophilic residues and sugars to the aqueous cyloplasmic or external regions. As a force is applied to displace the receptor in a direction normal to the membrane, it will tend to expose hydrophobic residues to water or hydrophilic ones to lipid, and hence to increase the free energy. Thus, in order to estimate the force (as the rate of change of free energy with distance) the amino acid sequence of the protein and its conformation near the membrane must be known. For one integral protein, glycophorin, a major constituent of the red cell membrane, both the amino acid sequence and its position in the membrane are known (*53*).

To estimate the free energy change as a molecule of glycophorin is pulled

through the membrane, I have neglected any folding of the portions of the molecule that are exposed to either the lipid or the cytoplasmic environment. For the portion buried in the lipid there are only about 27 residues spanning a distance of about 4.0 nm, so that little folding could be accommodated. In the cytoplasmic region there are approximately 40 residues, and it may be less legitimate to neglect their folding. I will return to this problem, but for the present I assume that displacement of a hydrophobic residue from the lipid to the aqueous environment gives the following free energy changes (*54–56*) in kilocalories per mole: Pro, 2.0; Phe, 2.5; Ile, 2.0; Tyr, 2.3; Leu, 1.8; Val, 1.5; and Met, 1.3. In addition, displacement of a polar residue (Asp, Glu, Arg, or Lys) from water to lipid is assumed to cost 5 kcal/mole (*57*). I have ignored the energy costs associated with the transfer of other amino acids, although it could be argued that the six serines in the cytoplasmic tail should be treated as strongly hydrophilic, rather than linked with each other by hydrogen bonds. Using these numerical values, I find that it costs about 20 kcal/mole to transfer residues 65 to 92 from the lipid into the extracellular water, and about 35 kcal/mole to transfer the cytoplasmic tail into the lipid. A further displacement of the receptor, such that the tail enters the extracellular aqueous region, will be energetically favorable. I thus conclude that there is an energy barrier against pulling the glycophorin molecule through the membrane which amounts to ~ 60 kcal/mole = 2.6 eV per molecule. If it is also assumed that this energy represents work done by a force in moving the receptor through a lipid bilayer of thickness 4 nm, the force required is, from Eq. 15, about 1.0×10^{-5} dyne per molecule. This is close to the estimated value of the force f_0 needed to rapidly break a specific ligand-receptor bond.

Thus the force required to pull an integral membrane protein out of the lipid bilayer is of the same order of magnitude as that required to rapidly break an antigen-antibody bond. Of course, most receptor-ligand bonds are readily reversible. However an integral membrane protein such as glycophorin is not about to pop out of the membrane spontaneously. This is because the energy barrier to translocation of the protein is far higher than that of the receptor-ligand bond. A relatively modest force serves to displace the receptor only because the distance over which it can act is relatively large, namely the thickness of the lipid portion of the bilayer. In an earlier section it was found that a critical force per bond of $\simeq 1/3\ f_0$ was sufficient to separate two cells. However, as can be seen from Eq. 17, a similar reduction of f_0 will not suffice to pull out a receptor. This is because the energy barrier is now so large compared to kT that the binding of the receptor in the membrane is essentially irreversible.

These estimates were made for a particular integral protein, and the results might be rather different for other receptors. In addition, the estimates were highly simplified; in particular, I neglected folding of the cytoplasmic tail. A folded tail would probably have all its polar residues exposed and would be even harder to pull through a membrane. Displacement of lipids might then be more likely than simple extraction of the protein. In addition, some receptors may interact with cytoplasmic proteins or with the cytoskeleton and thereby further resist being pulled out. For example, glycophorin interacts with the internal protein spectrin (*58*).

Some receptors may not be glycoproteins. For example, a ganglioside—a lipid molecule with an oligosaccharide headgroup—is the receptor for cholera toxin (*59*). The force required to pull a ganglioside or phospholipid out of the membrane can be estimated in the same manner. As before, the free energy change (for transfer of lipid from lipid to water) is divided by the distance over which the force can act. If the free energy change is taken as 15 kcal/mole (*54*), as determined for a phospholipid containing two 15-member hydrocarbon tails, and the distance is taken as 2 nm, the force required, from Eq. 15, is 5×10^{-6} dyne per molecule. This result suggests that it is about as easy to pull a ganglioside or a phospholipid out of a membrane as to break ligand-receptor bonding.

It thus appears that (depending on the details of the receptors involved) pulling receptors out of the membrane may be competitive with breaking ligand-receptor bonds. Although it is probably difficult to pull out a well-anchored integral protein, one could imagine various mechanisms, such as enzymatic clipping of cytoplasmic tails, that could modulate receptor anchorage and thereby adhesion (*41*).

Comparison with Experiments

In principle, the theoretical framework that I have developed could be used to interpret a vast number of experiments. However, in practice it is difficult to make quantitative comparisons. In some cases, such as those involving the natural adhesion of cells to surfaces, there is little information on the molecular species and their interactions that mediate the adhesion. In other cases, where the adhesion is known to be mediated by lectin or antibody molecules, the experiments were not designed to facilitate comparisons with theory. In most agglutination or binding assays, it is difficult to estimate the rates of collision of cells with each other or with surfaces, and the duration of the collisions in which binding is to take place is also uncertain.

To elucidate some of these considerations I will compare the theory with measurements of the binding of cells to lectin-coated fibers or to lectin-coated cells on fibers (*48, 49*). In one set of experiments, the binding of various cells to lectin-coated fibers was studied for lectin densities ranging from ~ 200 to 2×10^4 molecules per square micrometer (*49*). The cells were shaken in a medium exposed to the fibers at 80 cycles per minute with an amplitude of $\simeq 4$ cm. It follows that the peak fluid velocity relative to the fibers is $\simeq 20$ cm/sec, so that according to Eq. 24 a cell must establish about 300 bonds to the fiber in order to remain bound. The actual fluid flow is complicated and more precise quantitation is difficult.

Cells are found to adhere only to the sides of fibers that are normal to the plane of motion. This is presumably because a grazing cell-fiber collision is too brief to permit adequate bond formation; at a velocity of 10 cm/sec, a cell will travel its own diameter ($\sim 10\ \mu$m) in $\sim 10^{-4}$ second. The maximum rate at which bonds can form when cell and fiber are close together can be estimated from Eq. 13, with $N_1 = N_f$, the fiber surface density of lectin sites that bind saccharides, and N_2 the number of complementary saccharides on the cell surface per unit area. If the cells are fixed their receptors are immobile and according to Eq. 8 k_+ will be zero, so that no adhesion is anticipated, in accord with observation. However it should also be noted that erythrocytes, which have relatively few and immobile receptors, can be agglutinated if care is taken to minimize the mechanical forces tending to disrupt the aggregates (*60*).

The rate constants for binding of concanavalin A to sugars or to erythrocytes are quite low: $k_+^s \sim 10^4$ to $10^5 M^{-1}$ sec^{-1} (*60, 61*). This is far smaller than the diffusion limit d_+^s, so that from Eq. 8 $k_+^s \simeq Dr_+ \simeq r_+$, since $D \simeq 1 M^{-1}$. In addition, the diffusion coefficients for concanavalin A receptors are quite small, 4×10^{-11} cm^2/sec or less, as measured

on fibroblasts and myoblasts (*32, 34*). Therefore despite the small value of r_+, the membrane-bound reaction rate k_+ may be within an order of magnitude of its diffusion limit, say between 10^{-10} and 10^{-11} cm^2/sec. Taking the smaller value, $k_+^m = 10^{-3}$ μm^2/sec, and $N_2 \simeq 10^4$ μm^{-2} (*62, 63*), the maximum rate of bond formation will be $dN_b/dt \sim 10^{-2}$ N_1/msec. Thus if $N_1 \sim 10^4$ μm^{-2}, a near-maximum value, $dN_b/dt \sim 10^2$ μm^{-2} msec^{-1} and any cell-fiber collision for which the product of contact area and duration exceeds 3 μm^2 msec has a good chance of leading to adhesion. Similarly for $N_1 \sim 10^2$ μm^{-2}, the product must exceed ~ 300 μm^2 msec. As collision durations of ~ 1 to 10 msec and contact areas of ~ 1 to 10 μm^2 seem reasonable, it would seem that these figures are of the right order of magnitude. However without an understanding of the statistics of cell-fiber collisions, it is not possible to make quantitative comparisons between theory and experiment.

Once the cells had become bound to fibers, the reversibility of the binding by free sugars was examined (*49*). Binding by divalent lectins could be reversed by competitive free sugar at 0.001 to 0.01M. For an equilibrium constant of $10^6 M^{-1}$, the model shown in Fig. 5 predicts that these concentrations would reduce the equilibrium number of bonds by factors of 10^6 and 10^8, respectively, so that reversal of binding would be expected. For tetravalent concanavalin A, even at 0.3M the competitive saccharide α-methyl mannopyranoside failed to dislodge the cells. This may be because of the greater stability of intramolecular bonds mediated by a tetravalent as opposed to a bivalent ligand.

In further experiments cells were first attached to fibers, and then the adhesion of other cells to the fiber-bound cells was studied when the fiber-bound or the other cells or both were coated with lectins (*48*). Since in this case the lectin is on the cell surfaces rather than just on the fibers, cross-linking of cell surface carbohydrates competed with aggregation. The most favorable conditions for aggregation are those that minimize the cross-linking, namely conditions under which the cells that carry the lectin have been previously fixed. When both types of cells are fixed almost no binding results because, as before, $k_+^m = 0$. Fixation of the cells that do not carry the lectin decreases aggregation by decreasing receptor mobility. All of these experimental results are in agreement with the theory.

At high lectin concentrations one would expect to find poor binding between lectin-coated cells, because of a scarcity of free receptors for forming intercellular bonds. Such experiments could not be readily done with fiber-bound plus free cells because the free cells would tend to aggregate spontaneously if exposed to concanavalin A. However in other studies of the aggregation of erythrocytes (*64*) or hepatoma cells (*65*) by concanavalin A it was found that agglutination is much diminished by free concanavalin A concentrations $\gtrsim 10^{-6}M$. This is to be expected for $K^s \sim 10^6 M^{-1}$, as deduced by the authors (*64*).

Countless studies have indicated that transformed fibroblasts are more readily agglutinable by lectins than are their normal counterparts (*12, 13, 62*). Although some of this effect may be due to different receptor densities (*63*), increased mobility of receptors on transformed cells would also be an explanation [for example, see (*49*)]. In particular, the normal cells express LETS glycoprotein on their surface, which binds concanavalin A readily and is very immobile (*34*). Thus LETS-coated (normal) cells would be expected to be agglutinated less readily by concanavalin A and other lectins than non-LETS-coated (transformed) cells.

Discussion

In this article I have developed a theoretical framework for the analysis of adhesion that is mediated by bonds between specific molecules. The basic ideas are very simple and are essentially twofold. First, from a knowledge of the reaction rates for reactants in solution together with the diffusion constants of the reactants in solution *and* on membranes, the reaction rates for reactants bound to membranes can be estimated. Second, force equals energy divided by distance, and this can be used to deduce from microscopic bond properties the macroscopic forces required to separate cells. I have shown that the adhesion that is mediated by specific bonds can be strong compared to the expected nonspecific electrical forces between cells. Also, the force that is required to extract a receptor molecule from the cell membrane may be comparable to that required to break an antigen-antibody or lectin-carbohydrate bond.

I passed rather casually over many details and complications in the models. In some instances it would have been possible to present somewhat more precise or general formulations. For example, I could have considered the dependence of cross-linking and bridging rates on ligand length (*66*) or on the number of binding sites per ligand molecule (*67*). In other cases there are biological uncertainties that hinder confident application of the theory. For example, cross-linking of receptors may modulate the cell surface properties, including the receptor mobilities, in important ways. Nevertheless, by comparing model predictions with experimental results it may be possible to learn something about the rates and significance of modulation.

Although there have been countless experiments concerned with cell adhesion or agglutination, most of them cannot be quantitatively compared with the present theory. I hope that the existence of a theoretical framework will stimulate new experiments that are designed for such comparisons. In addition, it may be possible to reexamine past experiments and to find such parameters as collision rates and durations, which are required for application of the theory.

Several nontrivial extensions of the theory appear particularly worthwhile. One would be an analysis of the response of intercellular bonds, or of cell-to-surface bonds, to shearing forces, which are probably the forces most commonly involved in breaking up cell aggregates or dislodging cells from surfaces. Deformation of the cells should be included in this analysis. Another extension would be to examine the motion of cells on adhesion gradients (*68*), which may be important in embryonic development or for the understanding of cell-sorting experiments (*69*). I suggest that the proposed framework will furnish a new and fruitful approach to these and other important biological problems.

Note added in proof. In an earlier section, the possibility was raised that when a force is applied tending to remove an integral membrane protein from the membrane, the result may be to remove surrounding lipids together with the protein rather than to pull the protein through the intact lipid bilayer. In order to make a rough estimate of the force required to displace the lipids, let us suppose that a small cylindrical bilayer plug of radius r and thickness h is displaced, together with the protein, from the membrane. An area of lipid $\sim 2\pi rh$ is thereby exposed to the aqueous extracellular environment and the associated free energy change is about 30 cal/mole-Å^2 (*70*). Hence the free energy change is about $60\pi rh$ cal/mole per plug. This lipid area may become exposed when the plug is displaced by a distance $\simeq h/2$ so that the required force is about $120\pi r$ cal/mole-Å $= 2.6 \times 10^{-6}$ r dynes, where r is measured in angstroms. For example, if $r = 10$ Å,

$f \simeq 2.6 \times 10^{-5}$ dynes, which is somewhat larger than the force estimated earlier for extraction of a glycophorin molecule. However, since the forces are estimated to be the same order of magnitude, we conclude that some integral membrane proteins, especially those with folded cytoplasmic tails, may preferentially come out of the membrane together with associated lipids rather than in naked form.

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56. Abbreviations: Pro, proline; Phe, phenylalanine; Ile, isoleucine; Tyr, tyrosine; Leu, leucine; Val, valine; Met, methionine; Asp, aspartic acid; Glu, glutamic acid; Arg, arginine; and Lys, lysine.
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NEWS AND COMMENT

A Bright Solar Prospect Seen by CEQ and OTA

The Sun Day observance of 3 May was intended as more than a good-humored celebration of the sun and the promise of solar technology. It was also expected to afford the opportunity for some strong criticism of what many solar advocates see as the government's failure to push the development and commercialization of this technology more vigorously.

Indeed, such criticism has been brewing for some time. Several national environmental groups recently accused the Carter Administration of backing away from a commitment to energy conservation and development of decentralized, renewable energy systems in favor of nuclear power and massive subsidies for synthetic fuels.

As it happens, new reports by the Administration's own Council on Environmental Quality (CEQ)—which under President Carter has been allowed to play somewhat the role of a gadfly—and the congressional Office of Technology Assessment (OTA) lend credence to the view that federal support for solar development has not been nearly in keeping with this technology's promise. Both reports say that the technology is on the verge of a flourishing new phase but that, without aggressive federal support, its contribution to national energy supplies will fall far short of what now seems technically and economically achievable.

The CEQ report, based on an extensive literature review and discussions with solar scientists, says that it should be possible for solar technology to supply a quarter of all U.S. energy by the year 2000 and "significantly more than half" by 2020. "For the period 2020 and beyond, it is now possible to speak hopefully, and unblushingly, of the United States becoming a solar society," the CEQ adds. No federal agency has ever previously held out even the possibility of so rapid a growth of solar energy, and

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