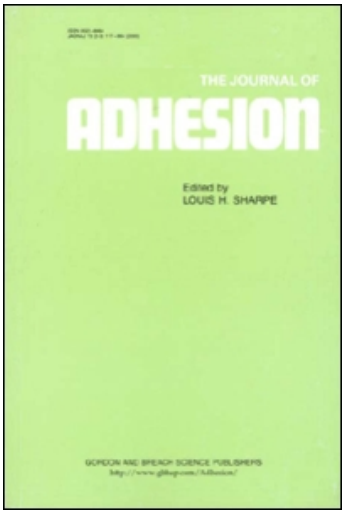

The Journal of Adhesion
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453635

Some Features of Physical Forces Between Biological Cell Membranes

V. A. Parsegian[a]; David Gingell[ab]
[a] Department of Health, Education and Welfare, Physical Sciences Laboratory, Division of Computer Research & Technology, National Institutes of Health, Bethesda, Maryland, U.S.A. [b] Department of Biology as Applied to Medicine, Middlesex Hospital Medical School, London, England

To cite this Article Parsegian, V. A. and Gingell, David(1972) 'Some Features of Physical Forces Between Biological Cell Membranes', The Journal of Adhesion, 4: 4, 283 — 306
To link to this Article: DOI: 10.1080/00218467208075010
URL: http://dx.doi.org/10.1080/00218467208075010

J. Adhesion, 1972, Vol. 4, pp. 283–306

Printed in Northern Ireland

Some Features of Physical Forces Between Biological Cell Membranes

V. A. PARSEGIAN and DAVID GINGELL †

Physical Sciences Laboratory, Division of Computer Research & Technology, National Institutes of Health, Department of Health, Education and Welfare, Bethesda, Maryland 20014, *U.S.A.*

(*Received March* 14, 1972)

We have applied several advances in the theory of electrostatic and electrodynamic (van der Waals) forces to the problem of biological cell adhesion. Long-range interactions (i.e., those acting across separations much greater than interactomic distances) are strong enough to hold cells together or to artificial substrates. There is a wide range of attractive energies depending on the interacting substances, in particular a ten-fold range with the artificial materials and an energetic specificity between cells of like type to allow a population of mixed cell types to aggregate with likes sticking to likes (as is commonly observed experimentally).

The present physical approach can provide a useful logic for designing techniques to probe the cell surface and points out several hitherto neglected aspects of the cell surface germane to the study of cellular adhesion.

1 INTRODUCTION‡

The rich diversity of adhesion properties exhibited by biological cells appears to require theoretical analysis beyond the usual scope of adhesion science. Cellular processes involved in cell division, growth, differentiation, and embryological development are dynamic events intimately associated with changing cell contact. Yet physical adhesion phenomena are generally

† Present Address: Department of Biology as Applied to Medicine, Middlesex Hospital Medical School, Cleveland Street, London, W1, England.

‡ Most of the present text is a shortened version of a paper presented at the Symposium on Recent Advances in Adhesion during the 162nd National American Chemical Society Meeting, September, 1971. The full text will appear in the volume "Recent Advances in Adhesion", ed. L. H. Lee, Gordon & Breach Science Pub., New York & London, 1972.

understood in terms of a static picture. While much experimental information is still fragmentary, two broad features of cellular interactions can be recognized.

There is evidence of cell contact specificity such that dissociated cells of varied tissue types will preferentially associate with cells of like type rather than with cells from other tissues[1-3]. This selectivity may be lost upon cancerous "transformation" to cells capable of invading populations of normal cells. Specific association[4,5] and cancerous behavior[6-9] are influenced by material on the cell periphery. Enzymatic removal of these outermost materials can modify cellular adhesive contact[10,11]. Cells removed by force from contact with artificial substrata leave behind an adsorbed layer which appears to be cell surface material[12-15]. This would indicate stronger adhesion of the extracellular layer to an artificial substrate than to the cell itself, as stressed by Weiss[16].

Conversely, cells can exhibit considerable generality of adhesion both to cells and to materials such as glass, metals and plastics with which a common covalent bonding mechanism is impossible. Free-living amoebae, for example, show a remarkable faculty to stick to almost any material yet paradoxically do not seem to adhere to each other. Intercellular adhesions can form between cells from different tissues[17,18].

Physical considerations of contact between biological cells have been largely unsatisfactory, both because of limited theoretical methods and a paucity of experimental information. By exploiting recent advances in the physics of attracting material bodies[19-24] one can now compute the interaction between two cells or between a cell and a substratum as the bodies are brought together from large separations. Following Bangham and Pethica[25] and Curtis[26,27], we shall consider two kinds of interaction: electrostatic (Coulombic) forces which are repulsive between identical surfaces and electrodynamic (electromagnetic or van der Waals†) forces which are attractive between like surfaces.

Cells typically bear acidic groups that dissociate to give negative electrostatic charge distributed around the cell periphery. The potential due to this charge depends upon the dissociation characteristics of the acid groups, their spatial distribution, and the ionic composition of the suspending medium. The required equations for these parameters have been developed elsewhere[28-31].

In order to compute the attractive electromagnetic forces it is necessary to eschew the prevalent assumption that electrodynamic energies of interaction between large bodies are simply the sum of inverse sixth power interactions

† In this paper we use "electrodynamic" to cover the terms "van der Waals" and "electromagnetic".

between their atoms. Rather we follow the fundamental and rigorous approach of Lifshitz[32] and of Dzyaloshinskii, Lifshitz and Pitaevskii[33] as developed by Parsegian and Ninham[19–24]. Exact expressions for the electrodynamic energy can be derived for separations greater than interatomic distances by summing the oscillator energies of all electromagnetic fluctuations extending over the whole structure. Experimental information for evaluation of the electromagnetic energy is available, in principle, from absorption spectra and refractive indices of the component materials.

The physical analysis described in this paper furnishes three main categories of results.

First, we are able to make several conclusions relating the current picture of cell membrane structure to consequences of that structure in cell-cell interaction.

a) At long distances cells experience a non-specific mutual attraction due primarily to the thin lipid membrane bounding the cells.

b) The mucoprotein "fuzz" peripheral to the lipid can exert a specific attraction at short distances <50 Å where fuzz-fuzz interaction dominates the electrodynamic energy. Herbert Jehle[34,35] has emphasized the intrinsic specificity of van der Waals forces in relation to cell specificity in the limit of cell contact. Our analysis reveals a progressive expression of such specificity as two cells approach each other.

c) There is a relatively weak local "secondary" energy minimum, $\sim 5 \times 10^{-4}$ erg/cm^2 deep, at 50 to 80 Å separations.

d) Strong "primary" energy minima of the order of 0.1 erg/cm^2 may occur in the limit of close cell contact, but details of this contact are beyond the applicability of the present model.

Second, we are able to consider the cell suface fuzz material in terms of experimentally amenable features usually neglected in biological studies:

a) Since electrodynamic forces are now calculable from absorption spectra it is imperative that the spectral features of the cell peripheral fuzz be measured. In particular, spectra in the near-to-mid-ultraviolet region appear to be of importance in understanding specific intercellular adhesion.

b) The composition, distribution and weight density of cell fuzz, even stated as average quantities, are more important to a first calculation of cell attraction than are the conformation properties of membrane protein fractions.

Third, interactions of cells with a number of inert substrata, including metals, quartz and plastics, have been tentatively modeled. The interaction energies depend on the spectroscopic properties of the substrata as well as those of the cell periphery. The vastly different attractive energies predicted

on the various substrata suggest possible biomedical applications. We also consider experimental approaches based on force-energy computation utilizing parameters which determine these forces as experimental variables.

We shall next describe the model cell boundary used as the basis of our formulation. The Results section reports numerical estimates for some cell-cell and cell-substratum interactions. In the Discussion we attempt to relate these results to experimental studies on cell contact and discuss experimental paths suggested by analysis.

2 METHODS

Models

The model that we use for the cell periphery is the "unit membrane" or Davson-Danielli bilayer. This is a bimolecular leaflet or lipid molecules merged to form a planar hydrocarbon slab 40 Å thick possibly coated on one or both sides by a layer of wet protein and saccharide materials. This outer "fuzz" layer bears fixed electrostatic charge which sets up an electric double layer in the vicinity of the membrane. This region exhibits a wide range of thicknesses, mass and charge densities from cell type to cell type. In the absence of full experimental data we assign the properties of a liquid hydrocarbon to the inner core membrane and those of a highly concentrated saccharide solution to the fuzz layers. Unless otherwise stated we use thicknesses of 40 Å and 20 Å respectively for hydrocarbon and fuzz layers.

The cell periphery is in ionic equilibrium with a solution containing 0.145 M monovalent salt and 0.002 M divalent cation.

Our tentative picture of the interaction of two membranes is given in Figure 1.

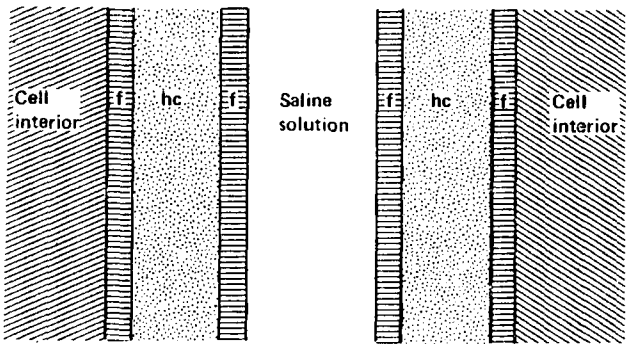

FIGURE 1 Geometric scheme for the interaction of two fuzz-coated cell membranes across salt solution. f = fuzz, hc = hydrocarbon, m = saline solution. In computations regions f and hc are assigned thicknesses 20 Å and 60 Å respectively unless the contrary is stated.

Assumptions made about the properties of the cell interiors or the fuzz layer facing the cell interior have negligible influence in the numbers calculated below. On the other hand the thickness, weight density, and electrostatic charge density of the outer fuzz layers and the ionic properties of the intervening physiological saline are of critical importance.

The geometry of one cell interacting with a planar substratum is similarly shown in Figure 2.

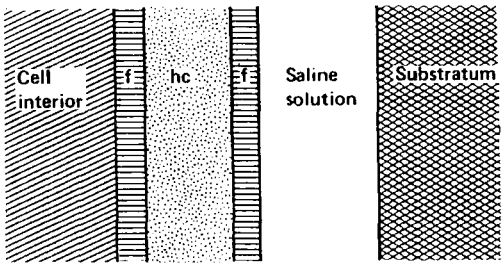

FIGURE 2 Scheme for the interaction of one fuzz-coated cell membrane with a substratum across salt solution.

We use a planar geometry because cells in close interaction tend to flatten out to make contact with each other or with a planar substratum. Most of what we shall say will deal with the range $l \lesssim 200$ Å $= 0.02$ micron. This is less than the ~ 0.1 micron radii of curvature sometimes observed for cell surface extensions It would probably be possible to extend the rigorous formulation of interaction energies to non-planar geometries but this is most tedious and unlikely to affect the physical principles revealed by much simpler planar analysis.

The equations employed for the present computations are described elsewhere. Van der Waals electrodynamic interaction formulae are given in reference 22 while the relevant material properties are derived in references 36, 37, and 38. Electrostatic interactions are derived in references 29, 30, and 31.

3 RESULTS

Electrodynamic interactions between cells and inert substrata

Figure 3 shows electrodynamic (ed.) energy versus distance separating a model cell surface from a variety of substrata. As in all calculations, the surfaces are considered flat. A corresponding curve for cell-cell interaction (or membrane-membrane interaction, terms used synonymously here) is

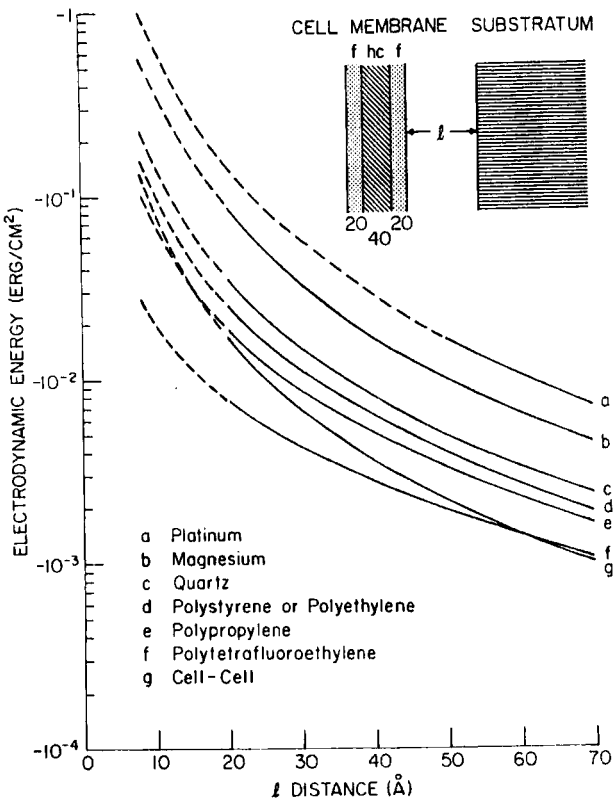

FIGURE 3 Curves a–g show attractive electrodynamic energy versus distance separating cell membrane from various substrata. Curve g is energy of cell–cell interaction for comcomparison. Note ten-fold range of energies. Dashed lines indicate regions where we think, the present continuum model for electrodynamic interactions is not accurate.

included for comparison. The magnitude of the attractive energy varies from greater than 16×10^{-3} erg/cm^2 down to 2×10^{-3} erg/cm^2 at 50 Å separation. Attractive forces at this distance (in dyne/cm^2) are approximately:

cell-polytetrafluoroethylene	0.7×10^4
cell-cell	1.0×10^4
cell-polypropylene	1.5×10^4
cell-polyethylene	1.8×10^4
cell-polystyrene	1.8×10^4
cell-quartz	2.4×10^4
cell-magnesium metal	5.5×10^4
cell-platinum metal	$>9.0 \times 10^4$

The forces are in the same order as the energies and like the latter exhibit a ten-fold range of magnitude. Metals exert the strongest attraction, despite the fact that our formulation underestimates the attraction for materials of specific gravity exceeding unity[36]—probably a small error for magnesium (1.7) and quartz (2.2) but serious for platinum (21.45). Quartz comes between metals and polymers. The latter exert much weaker attraction while cell-polytetrafluoroethylene (PTFE or Teflon) interaction is singular in our list, being weaker than intercellular attraction at distances less than 60 Å. The order of attraction changes at large separations as electromagnetic retardation begins to affect the interactions differently, cutting down ultraviolet contributions to the energy[23]. In reference 36 we give an analysis of the changes in ed. forces with spacing for the several substances considered.

Electrodynamic interactions between cells

In order to examine the role of the membrane fuzz layer we next compare the cell-cell electrodynamic attraction calculated for four different assumptions about the fuzz. We first give (Figures 4a, b) the calculated energies for two

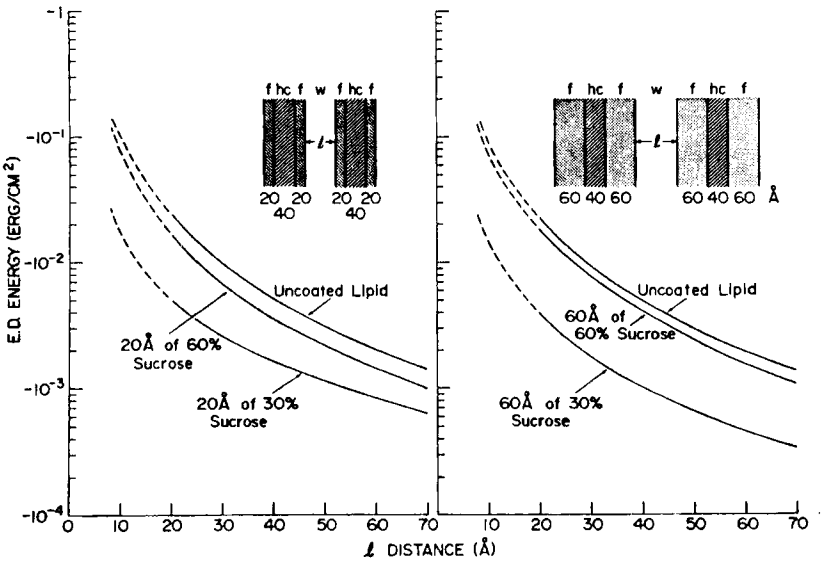

FIGURE 4 (a) Electrodynamic energy versus membrane separation. Curves for pure hydrocarbon membranes as well as membranes with 20 Å sugar coats are shown. Curves are dotted where the physical model may be unreliable.

FIGURE 4 (b) Interactions as in Figure 6a except that the hydrocarbon has 60 Å sugar coats.

assigned fuzz thicknesses, 20 Å and 60 Å, and for two assumed concentrations, 30% and 60%, of aqueous sucrose modeling the fuzz. In each case we relate the energies to those for interacting lipid membranes where the fuzz thickness is zero.

For the figures used here the saccharide layer lowers the attractive energy relative to lipid with no fuzz layer. The 60% concentration of sucrose increases attractive energy over that at 30%. The differences in energy for different coat thicknesses, but not composition, are lost for very small separations (compare for example Figures 4a and 4b, l = 20 Å and 60 Å of 30% sucrose: the energies are very similar). But at larger distances the dependence of energy on separation is clearly sensitive to coat thickness. At very long distances, several hundred Angstroms (not shown in the

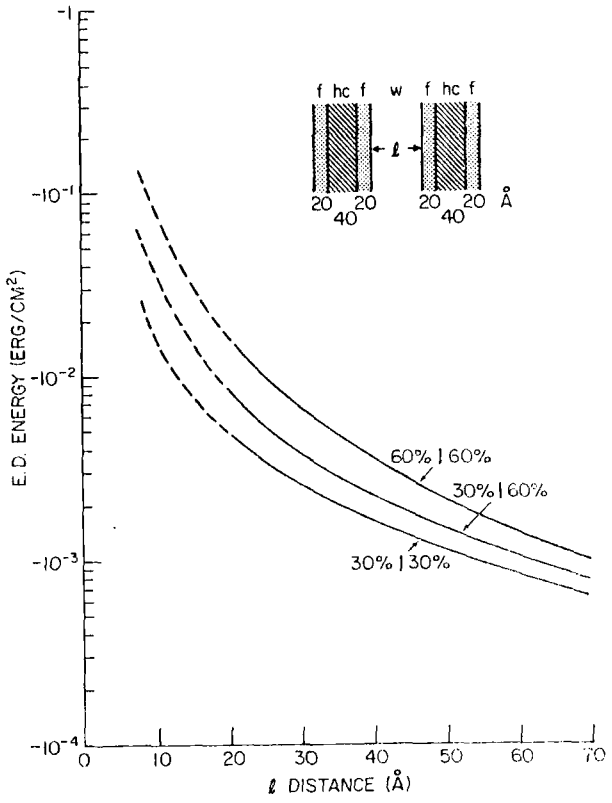

FIGURE 5 Electrodynamic energy versus membrane separation for different saccharide densities in the fuzz. Curves for interaction of membranes with 30% sugar and 60% sugar modeling the cell surface fuzz are given. Specificity of interaction is indicated (see text). Curves are dotted where the physical model may be unreliable.

figure), the interaction is dominated by the lipid-lipid attraction independent of the surface coat.

It is of interest that increasing the thickness of 60% sugar in the fuzz from 20 Å to 60 Å increases the interaction energy, whereas increasing the thickness of 30% sugar similarly decreases the energy. Thus it is possible that the density of fuzz could profoundly affect the qualitative behavior of interacting membranes. Discussion of this will be taken up in connection with cell reaggregation experiments.

The effect of surface coat sugar density is emphasized in Figure 5. Here we give electrodynamic energy versus distance curves for three cell-cell interactions where the fuzz is 20 Å but the sugar concentrations are 60% or 30%. We consider the interaction of membranes with like (60% | 60%) and (30 | 30%) or unlike (60% | 30%) membranes. The magnitudes of attractive energies are in the order 60 | 60 > 60 | 30 > 30 | 30. Further analysis reveals another inequality: the magnitude of 60 | 60 plus 30 | 30 interactions is greater than twice the 60 | 30 interaction. We will refer to this point in the Discussion in the context of cell attraction specificity.

Electrostatic interaction between cells

The electrostatic repulsive energy between apposing model membranes is shown in Figure 6. The fuzz thickness is set at 20 Å. The exponential energy functions give linear logarithmic plots with constant slope proportional to $-\kappa$ where κ is the Debye-Huckel reciprocal length. The value of the energy at $l = 0$ is the contact energy obtained as described elsewhere[30]. All curves are dotted as separations 20 Å $> l > 0$ indicating the region where the method is of doubtful validity. Energy at a given separation increases as the charge density in the fuzz increases. Curves for 200, 400, and 600 Å^2 per charge are given. It should be emphasized that the exponential decrease of electrostatic energy is accurate at distances >20 Å[29].

The electrostatic repulsive energy is moderately sensitive to the surface charge density. Doubling the area per charge from 200 Å^2 to 400 Å^2 reduces the repulsive energy by 64%. For example at $l = 50$ Å the interaction energy drops from 1.57×10^{-3} erg/cm^2 to 0.57×10^{-3} erg/cm^2.

The dependence of electrostatic repulsive energy on cell membrane fuzz thickness is illustrated in Figure 7. Fuzz thicknesses of 20 Å, 40 Å and 60 Å are used, with a constant area per charge of 400 Å^2. Interaction energies fall as the fuzz thickness increases. This is due to the higher population of mobile counterions allowed in the thicker fuzz layer: these counterions partly cancel the field due to fixed charge within the fuzz and consequently lower the electrostatic repulsion between layers. It can be seen that doubling the fuzz thickness from 20 Å to 40 Å decreases the repulsive energy by a

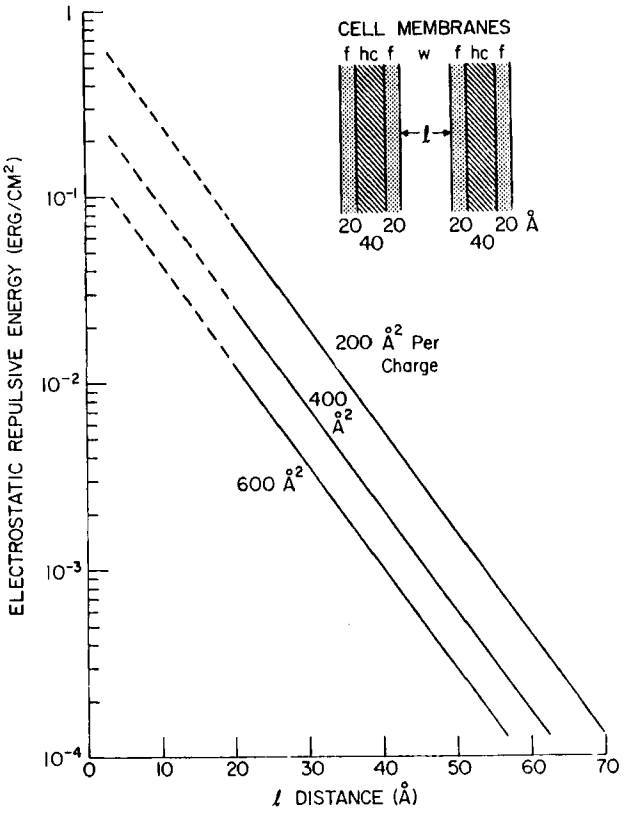

FIGURE 6 Electrostatic interaction energy (log scale) versus membrane separation l. Three curves for different fixed charge densities in the fuzz layer are given. Curves are dotted where the physical model may be unreliable. Note the exponential decay of electrostatic repulsion.

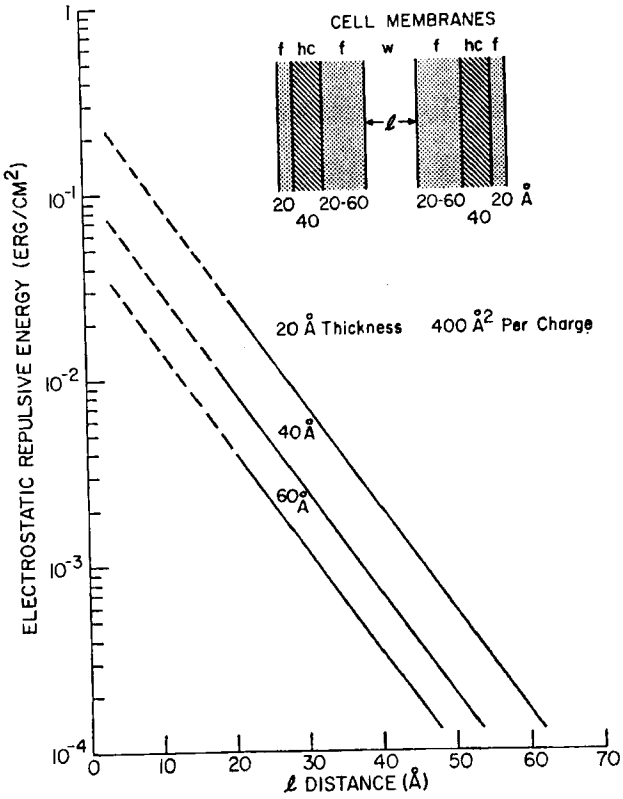

FIGURE 7 Electrostatic repulsive energy versus membrane separation. Fixed charge density is 400 Å²/ electron charge. Curves for different thicknesses as variables are given. The curves are dotted where the physical model may be unreliable. Note the inverse dependence on fuzz thickness.

factor of three. Tripling thickness from 20 Å to 60 Å decreases repulsion by a factor of six.

Combined electrodynamic and electrostatic interactions

Figure 8 shows both negative electrodynamic (ed.) energy and positive electrostatic (es.) energy plotted separately. Lines are dotted in the region of uncertainty. Where the absolute magnitude of the negative electrodynamic energy is equal to that of the positive electrostatic energy the curves intersect and the net energy is zero. These plots display this feature concisely. If es. and ed. curves cross twice the sign of the energy with decreasing net separation runs: negative, zero, positive, zero, negative and a local minimum

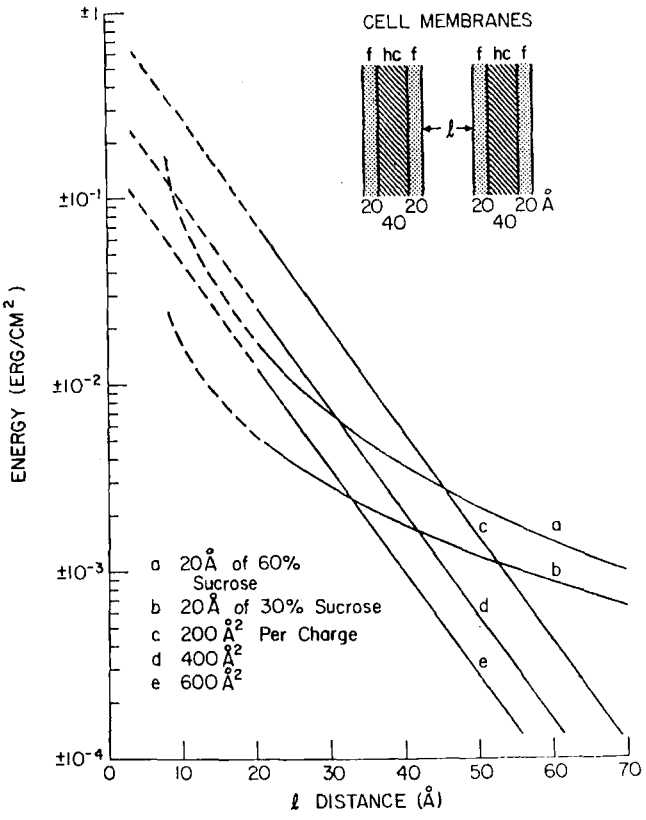

FIGURE 8 Superposition of curves for electrostatic (straight lines) and electrodynamic (concave up lines) energy from Figures 4a and 6. Crossing points indicate zero net energy of interaction.

exists at some separation greater than the larger zero energy position. If the ed. curve lies above the es. curve entirely the energy is negative at all separations. For example, interaction between identical membranes with 20 Å fuzz of 60% sugar having 400 Å^2 per charge results in a secondary minumum at $l > 31$ Å and a net energy maximum at a distance $8 < l < 31$ Å.

Figure 9 is similar to Figure 8 except that surface fuzz thickness is increased from 20 Å to 60 Å. The increase affects inner and outer layers but that

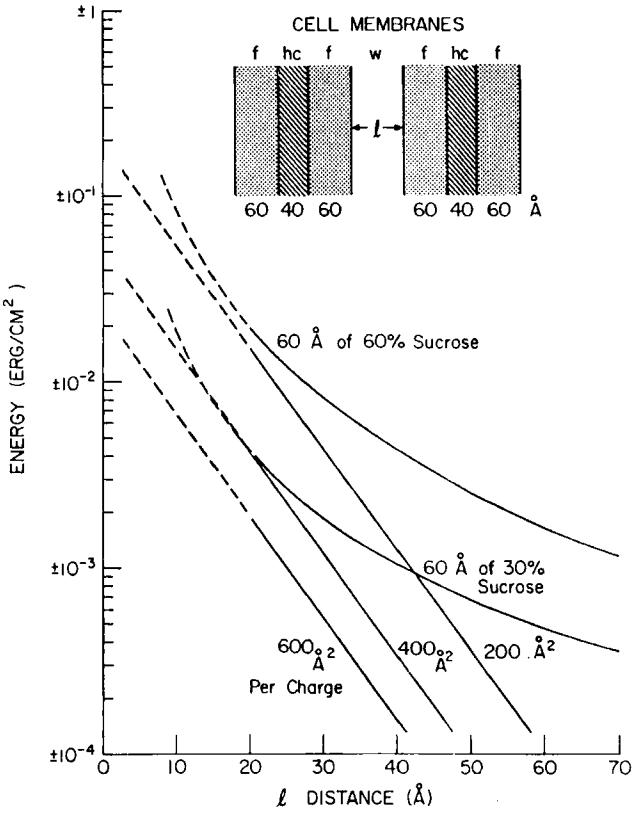

FIGURE 9 Superposed curves of electrodynamic and electrostatic energy. Electrodynamic data as in Figure 4b. Electrostatic curves for different fuzz charge densities shown.

inside (facing the cell interior) contributes very little to intercellular interaction. With reference to Figure 10 it can be seen that electrodynamic attraction is increased with increased fuzz thickness by 60% sugar but reduced by 30% sugar; a coat with greater attractive properties than lipid increases net attraction but a coat with smaller attraction decreases it.

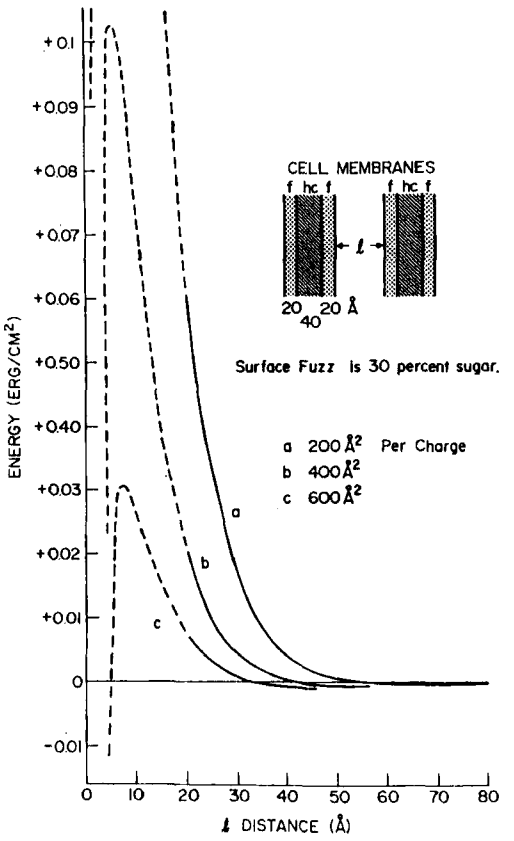

FIGURE 10 Total energy of membrane interaction. Data exactly as in Figure 8, but electrostatic and electrodynamic curves are summed rather than superimposed. Surface fuzz is represented by 30% sucrose.

Curve (a) is the sum of $b + c$ of Figure 8
Curve (b) is the sum of $b + d$ of Figure 8
Curve (c) is the sum of $b + e$ of Figure 8

Djstances of zero energy correspond to crossing points of Figure 8. The curves are dotted where the physical model may be unreliable.

The thick surface coat of 60% sugar thus results in a net attractive energy increase which predominates at all distances for 200 Å² per charge or lower densities. The thick coat of 30% sugar results similarly in a net negative energy for 600 Å² per charge at all separations but the electrodynamic and electrostatic curves intersect at 200 Å² and 400 Å² per charge, indicating regions of net positive energy.

Curves of summed electrostatic and electrodynamic energies for intercellular interaction are presented in Figure 11. A discussion of the elementary

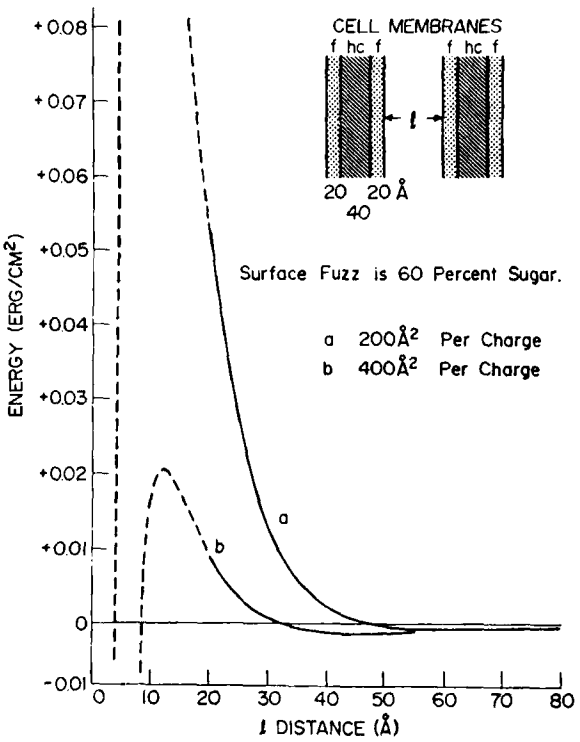

FIGURE 11 Total energy of membrane interaction. Data as in Figure 10 but showing summation of corresponding electrostatic and electrodynamic energies. Surface fuzz is represented by 60% sucrose.

Curve (a) is the sum of $a + c$ of Figure 8
Curve (b) is the sum of $a + d$ of Figure 8
The curves are dotted where the physical model may be unreliable.

properties of such force and energy curves has been given by Gingell[39]. The cell membranes have 20 Å of fuzz with 200 Å², 400 Å² and 600 Å² per charge. Fuzz is represented in the model by a 30% sucrose solution. Perhaps the most striking feature of the curves is the enormous repulsion at 5–20 Å when the area per charge is below 600 Å². Curves *a*, *b*, *c* show secondary minima at 49, 59 and 73 Å.

4 DISCUSSION

To what extent can a physical force model give a valid picture of interactions between the membranes of living cells? It is in the context of this question that we will discuss the foregoing calculations. While any satisfactory

understanding of cell contact in biochemical, immunological or physical terms is still distant, we believe that the present force-energy analysis is useful in at least three ways:

One, whatever the metabolic processes involved in cell-cell interactions, our calculations show that very strong attractive and repulsive forces operate purely by virtue of the structure and composition of the cell periphery.

Two, there consequently exists an important connection between studies of cell membrane structure, chemical composition and the calculation of intercellular forces. The connecting link lies in the spectroscopic characteristics of membrane components. Measurements of the principal absorption frequencies from the microwave through the ultraviolet, together with refractive index measurements in the same range, may suffice to characterize the components[23,37]. We have begun to understand how these physical properties of membrane materials play a dominant role in determining the forces of membrane contact and cellular behavior. Previous work on biological membranes has largely ignored the spectroscopic properties of the proteins and saccharides of the cell periphery. Emphasis has been on more detailed chemical analysis and on conformational aspects which may be of much less importance to intercellular forces. In the present treatment we have been forced to assume vastly simplified spectroscopic properties for membrane surface material because of the paucity of data.

Three, since experimentally amenable variables which govern intercellular forces can now be identified, it is possible to design experiments to examine the role of these forces in cell contact. For example, cell adhesion to artificial materials can be analyzed in terms of the spectroscopic properties of various substrata. Similarly, coating cells with known substances could be used to check predictions of the forces of intercellular adhesion.

Strength of interactions

A question of fundamental importance is whether the calculated attractive forces are strong enough to hold cells to other cells or to a substratum. The net energy curves can show two local energy minima—a weak "secondary" minimum at 40–80 Å separation and a stronger "primary" minimum predicted when cellular materials come into physical contact (<5 Å separation). We cannot decide on the basis of present information which of these minima is likely to be preferred in any particular case. Either of them is strong enough to maintain cell adhesion in the face of thermal energy. The depth of the secondary minimum is of the order of 5×10^{-4} erg/cm^2 while the primary minimum (assuming the dubious accuracy of our model at short distances) must be of the order of the surface energy, 0.1 erg/cm^2. For a cell contact area of only one square micron the net energy of interaction

will be 5×10^{-12} erg and 10^{-9} erg in secondary and primary minima respectively. If we compare this to the thermal energy of an unattached particle, which is of the order of $kT = 4 \times 10^{-14}$ erg, these energies are of the order of $10^2\ kT$ and $10^5\ kT$ respectively: more than enough to hold cells together.

The derivative force pulling the cells into a secondary minimum $\sim 10^3$ dyne/cm^2, is comparable to forces cells can exert. Indeed forces of protrusion 10^3 dyne/cm^2 can be exerted by amoebae[40] and contractile forces of 10^4 dyne/cm^2 can be exerted by cells in culture[41] suggesting that the calculated secondary minimum is not so deep that cells cannot break contact from it. In contrast, the repulsive force required to overcome the potential barrier to a primary minimum is of the order of 10^5 dyne/cm^2. An attractive force of similar or greater magnitude is likely to occur on the other side of the barrier, although our methods are unreliable at such short distances. There is some evidences[42-44] that forces of the order of 5×10^6 dyne/cm^2 are needed to pull epithelial cells apart by micromanipulation, suggestive of primary contact. Viscometric studies on red blood cells in plasma may imply very small forces, $\sim 10^{-7}$ dyne, required to separate cells, i.e. 10 dyne/cm^2 for contact $\sim 1\ \mu^2$ [45]. Plasma fibrinogen is believed to reversibly cross-link red cells, however similar forces are apparent from measurements in saline solution[46]. These low forces may possibly indicate sliding of cells past each other. Sliding forces several orders of magnitude lower than perpendicular separation are predicted[39]. Our calculated forces lie within the rather wide range of experimental values but these measurements do not provide a reliable test of our model nor indicate whether cells rest in primary or secondary minima.

No reliable measurements of cell-cell adhesion energies exist as yet (despite claims which range over a tenfold factor of disagreement) so it is not clear how to evaluate the calculated depth of energy minima except in comparison to thermal energies. Several measurements of cell surface tension have been reported; these cover a range of values over two orders of magnitude up to 0.1 erg/cm^2. If the energy of contact had to be expended to flatten and stretch the surfaces to meet each other, a correspondence between surface tension and contact energy would be possible. But ambiguity of "surface tension" as a descriptive parameter for the cell surface makes such a correspondence untenable.

Cell adhesion specificity

Cells are seen to associate with cells of like type to form well-defined tissues. Evidence for the adhesive specificity assumed to underlie this behavior has been inferred from many experiments (e.g. references 1, 2, 4 and 5) where

cells dissociated from several tissues are reaggregated in vitro. The cells apparently reaggregate into clumps of similar tissue type.

If this phenomenon is to be understood in terms of the contact energies between cells, a necessary thermodynamic condition for its occurrence is as follows (H. Jehle, *et al.*[34]): let G_{ij} be the energy of interaction between cells of types i and j. The condition that cells of types a and b will stick to their own kind (a-a and b-b associations) rather than unlike kind (a-b association) is the inequality,

$$G_{aa} + G_{bb} < 2G_{ab}$$

Here it is understood that the quantitites G_{aa}, G_{bb} and possibly G_{ab} are negative. Further the absolute difference

$$|G_{aa} + G_{bb} - 2G_{ab}|$$

must be much greater than thermal energy kT.

We have chosen to look at the energy G as a sum of attrative energy, G^{ed}, and repulsive energy, G^{es}, so that G can be broken up as

$$G_{ij} = G_{ij}^{ed} + G_{ij}^{es}$$

We can then look at properties of each of these terms, G^{es} and G^{ed}, in their effect on the inequality

$$G_{aa} + G_{bb} < 2G_{ab}$$

The dominant contribution to the attractive electrodynamic interaction G_{ij}^{ed} between two bodies i and j across substance w is proportional to a sum of products $(I \cdot J)$ such that

$$G_{ij}^{ed} \propto -\Sigma'(I \cdot J)$$

where the individual terms, I, J in the sum are due to polarizabilities of the materials at well-defined frequencies[22,32,33,36]. We can write

$$G_{aa}^{ed} \propto -\Sigma'(A \cdot A)$$
$$G_{bb}^{ed} \propto -\Sigma'(B \cdot B)$$
$$G_{ab}^{ed} \propto -\Sigma'(A \cdot B)$$

and consider the inequality in terms of the relation between the individual products $A \cdot A$, $A \cdot B$, and $B \cdot B$. We know from the relation between arithmetic and geometric means that for unequal A and B, for every term in the sum

$$-A \cdot A \; -B \cdot B < -2A \cdot B.$$

Consequently the total electrodynamic energy leads to sorting by satisfying

$$G_{aa}^{ed} + G_{bb}^{ed} < 2G_{ab}^{ed}.$$

The repulsive electrostatic interaction across an ionic solution between two planar surfaces of charge densities σ_1 and σ_2 is dominated by the form

$$G_{ij}^{es} = \sigma_1 \sigma_2 f(l)$$

where $f(l)$ is an exponential function of separation[31]. If the surface charges are of like sign (cells normally bear a preponderance of negative charge), then the repulsive energies G_{ed}^{es} between unlike bodies obey the relation

$$G_{ab}^{es} = \sqrt{G_{aa}^{es} G_{bb}^{es}} \quad \text{so that} \quad G_{aa}^{es} + G_{bb}^{es} > 2G_{ab}^{es}$$

Between positive quantities G^{es} the repulsive component of the total energy will favour the interaction of dissimilar bodies. The electrostatic energy may act to cause mixing of cell populations.

If two cells adhere spontaneously, it is evident that the net interaction energy is negative. In the present model, the negative electrodynamic energy must dominate electrostatic repulsion. This attractive energy is also a source of specificity. Since calculated net contact energies for one square micron contact area are greater than 50 kT per interaction, *differences of only a few percent in the electrodynamic attraction will suffice to satisfy the specificity inequality* $|G_{aa} + G_{bb} - 2G_{ab}| > kT$.

We have alluded to three variable properties of the surface fuzz alone that can give the necessary differences:

(a) fuzz coat thickness

(b) fuzz coat material density

(c) absorption spectra of fuzz proteins or saccharides.

For example, in Figure 5 we showed calculated interactions between cells bearing 30% or 60% saccharide in their fuzz coats. Assuming all cells have one negative ionic charge per 400 Å^2 and a fuzz thickness of 20 Å, we can compare the net energies $G_{30}|_{30}$, $G_{60}|_{60}$ and $G_{30}|_{60}$ at the secondary minima

$$\begin{aligned}\Delta G &= G_{30}|_{30} + G_{60}|_{60} - 2G_{30}|_{60} \\ &= -0.684 \times 10^{-3} - 1.623 \times 10^{-3} + 1.954 \times 10^{-3} \\ &= -0.353 \times 10^{-3} \text{ erg/cm}^2 \\ &\sim 88kT/\mu^2\end{aligned}$$

For contact areas of one square micron the magnitude of the inequality is about 88 kT, which is sufficient to overcome any entropy of mixing.

Our list of structural variables influencing specificity is only a beginning. There is no virtue in assuming that one particular variable is responsible for interaction specificity and then creating a model that "explains" why cells usually stick to their own kind. There are too many candidate variables.

Nor does this approach help to relate the many lock-and-key or signalling mechanism, favoured by biochemists and immunologists, to the physical forces which must underlie these mechanisms. Indeed there is not reason *a priori* to reject models of interaction that assume covalent bonding or antigen-antibody locking of cell to cell. The long-range attraction (i.e. at distances great compared to interatomic spacing) discussed here is manifested purely on the basis of probable membrane structure. It is a potential source of specificity but not necessarily the only one. The attachment of fuzz material to the cell surfaces might be caused by enzyme-mediated covalent bonding, by immunological short-range locking[1] electrostatic forces, long-range electrodynamic forces etc. Once formed, the membranes may then interact as we describe. In Figure 12 we sketch one scheme whereby the

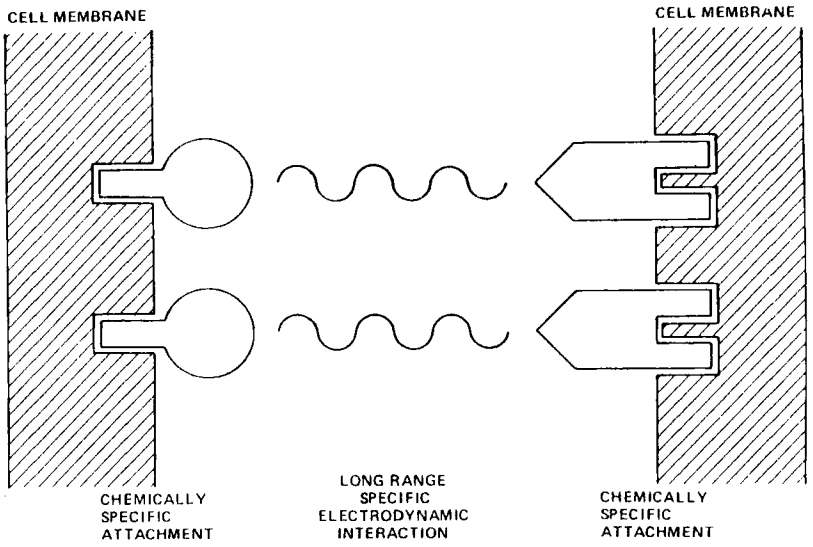

FIGURE 12 A possible model of membrane adhesion interaction specificity. Binding of surface glycoprotein is considered to be chemically specific for each type of membrane. Subsequent interaction between glycoprotein "fuzz' layers on different membranes exhibits electrodynamic specificity of a lower order.

binding of fuzz to membrane entails very specific lock-and-key fitting while less specific long range electrodynamic forces act to bring the membranes together. This scheme might fit nicely with that suggested by Roth, McGuire and Roseman[47] in their study of cell surface glycosyl transferases and with the requirement for cell surface saccharide synthesis prerequisite to cell adhesion as observed by Oppenheimer, Edidin, Orr and Roseman[48].

Modification of intercellular adhesion by univalent antibody binding[49] might also be considered in these terms.

A great deal of effort has been expended in isolating materials from cell surfaces which increase cell adhesiveness, as assayed by flocculation methods, when added back to cell suspensions[1,5,50]. Resynthesis of surface materials following removal by dissociation procedures facilitates adhesion[51]. Materials have been obtained from sponge cells which promote species-specific aggregation[52]. The substances are composed of protein and carbohydrate[50], but has probably not yet been isolated in a pure state. These sponge cell materials bind to the membrane surface with serological specificity[1]. Similar materials have been obtained from mammalian cells[53,54]. The latter work indicates tissue specificity of what is apparently a cell surface component.

Cell-substratum interactions

With regard to the design of synthetic materials to which cell adhesion could be controlled, the situation is relatively straightforward. For such interactions the language of physical forces and energies is most appropriate; evolution did not prepare the cell surface and cell enzymes to bind cells to polystyrene or PTFE by specific covalent bonds or intricate fitting mechanisms. Yet cells do adhere to a wide variety of artificial substances. The advantage in considering these materials is that, unlike the cell fuzz, they can be readily studied, characterized and designed. It may even be possible to create materials having attractive characteristics required for specific practical problems encountered in the development of surgical materials. Foremost among these is the problem of designing materials to which blood cells will not adhere[55–60]. A salient feature of our membrane-substratum calculations is the wide range of possible attractive energies. For one material, PTFE, there is even a repulsive component to the electrodynamic energy. Metals exert the strongest attraction because of the very high "polarizability" of conduction electrons.

In order to make the calculations of membrane-substratum interactions, it is necessary to use a rather strongly idealized description of the substratum electrodynamic properties because of limited present knowledge of the data. Since absorption spectra can be converted into estimates of electrodynamic forces there is good reason to measure spectra more carefully over the entire frequency range. Especially for ultraviolet frequencies, the existence of several absorption peaks, rather than one average peak that we assume, may lead to features of interaction seen only crudely so far. The greater the similarity between the absorption spectra of cell membrane and substratum compared to that of the intermediate substance, the greater will be the attractive force. The spectrum of the substratum material may also

provide a probe of the electromagnetic properties of the cell surface. We also have assumed that the substratum material is perfectly smooth and that the electromagnetic properties were that of the bulk material right up to the surface. For some materials, such as glass and some plastics, the surfaces are apparently so rough that it would probably be futile to try to relate measured adhesion energies to those predicted by the present calculations. Glass is also a poor candidate for measurement because metabolic events in adhesion[61] may be affected by ions leached out of the glass, obscuring physical factors governing cell adhesion. Furthermore, changes in the glass surface layer, which is very sensitive to chemical pretreatment[62–64], alter the spectroscopic properties of just that region vital in adhesion.

Synthetic materials such as polystyrene and PTFE can probably be made with chemically and physically well-defined surfaces and non-diffusible inner components. These materials are intrinsically superior to glasses for cell adhesion studies. For the same reason the attraction of cells to the interfaces of immiscible liquids[65,66] may provide advantages of smoothness and well defined material properties.

Actual measurements of cell-substratum interaction are fraught with the same ambiguities found in cell-cell interactions. Many conclusions rest on the assumed correlation of rates of adhesion with strengths of adhesion (for example: references 65 and 66). The complex media in which measurements are performed usually contain macromolecular components which coat the walls of the vessel[59,67,68]. There is frequently a question of whether the cell interacts with the material of the vessel wall or with adsorbed medium components that coat the wall. Forces other than those considered here may be important. One must consider charge on the substratum setting up an electrostatic double layer and the possibility of electrostatic image forces repelling or attracting the cell to a surface. There are too many things occurring simultaneously in the attachment or detachment of a cell sticking to a crudely defined surface to use cell contact measurements for information about long-range forces. It is possible and preferable to measure the equilibrium attractive force between a cell surface and a smooth interface held a finite distance from the cell. This enforcement of finite separation would put the measurement in the regime of the long-range force theory discussed here.

The distinction we make here between long-range attractive and repulsive forces and the regime of close contact may actually be useful in seeing the distinction between physical and biochemical or immunological views of cellular interaction. It is certain that long-range forces exist and that they are sufficiently strong to control cellular interactions. After these forces have acted to bring the cells together chemical reactions perhaps due to electrostatically triggered physiological responses of the cells to such

proximity[28,30,39,69–70] may further control close contact behaviour of cells. This close contact is properly the regime of immunology and biochemistry. We suspect that such a combination of physical and biological descriptions can give a fuller understanding of cellular interaction.

Acknowledgements

We are happy to point out the long collaboration of Barry Ninham with V.A.P. during which many of the physical methods used here were developed.
We thank Michael Edidin, Steven Oppenheimer and Herbert Jehle for many helpful comments; we appreciate the aid of Brenda Gingell and Valerie Parsegian in preparation of this text.

References

1. A. A. Moscona, *Devel. Biol.* **18**, 250 (1968).
2. M. S. Steinberg, *J. Exp. Zool.* **173**, 395 (1970).
3. J. P. Trinkaus and J. P. Lentz, *Devel. Biol.* **9**, 115 (1964).
4. A. A. Moscona, *Proc. Natl. Acad. Sci. U.S.* **49**, 742 (1963).
5. T. Humphreys, *Devel. Biol.* **8**, 27 (1963).
6. M. M. Burger, *Proc. Natl. Acad. Sci. U.S.* **62**, 994 (1969).
7. M. M. Burger, *Nature New Biol.* **231**, 125 (1971).
8. R. E. Pollack and M. M. Burger, *Proc. Natl. Acad. Sci. U.S.* **62**, 1074 (1969).
9. M. M. Burger and K. D. Noonan, *Nature* **228**, 512 (1970).
10. L. Weiss and D. L. Kapes, *Exptl. Cell Res.* **41**, 601 (1966).
11. L. Weiss, E. Mayhew and K. Ulrich, *Lab. Invest.* **15**, 1304 (1966).
12. L. Weiss and R. R. A. Coombs, *Exptl. Cell Res.* **30**, 331 (1963).
13. M. D. Rosenberg, *Biophys. J.* **1**, 137 (1960).
14. G. Poste and L. W. Greenham, *Cytobios.* **2**, 243 (1970).
15. G. Poste and L. W. Greenham, *Cytobios.* **3**, 5 (1971).
16. L. Weiss, *The Cell Periphery Metastasis and Other Contact Phenomena* (Elsevier, Amsterdam, 1967).
17. A. S. G. Curtis, *J. Embryol. Exp. Morph.* **23**, 253 (1970).
18. A. S. G. Curtis, *Nature* **226**, 260 (1970).
19. V. A. Parsegian and B. W. Ninham, *Nature* **224**, 1197 (1969).
20. V. A. Parsegian and B. W. Ninham, *Biophysics J.* **10**, 664 (1970).
21. V. A. Parsegian and B. W. Ninham, *J. Coll. & Int. Sci.* **37**, 332 (1972).
22. V. A. Parsegian and B. W. Ninham, *J. Theor. Biol.*, in press.
23. B. W. Ninham and V. A. Parsegian, *Biophys. J.* **10**, 646 (1970).
24. B. W. Ninham and V. A. Parsegian, *J. Chem. Phys.* **52**, 4578 (1970).
25. A. D. Bangham and B. A. Pethica, *Proc. R. Soc. Edinburgh*, **28**, 43 (1960).
26. A. S. G. Curtis, *Biol. Revs.* **37**, 82 (1962).
27. A. S. G. Curtis, *The Cell Surface: Its Molecular Role in Morphogenesis.* (Logos Press, Academic Press Inc., London, 1967).
28. D. Gingell, *J. Theor. Biol.* **17**, 451 (1967).
29. B. W. Ninham and V. A. Parsegian, *J. Theor. Biol.* **31**, 405 (1971).
30. V. A. Parsegian and B. W. Ninham, to be published.
31. V. A. Parsegian and D. Gingell, to be published.
32. E. M. Lifshitz, *Zh. Eksp. Teor. Fiz.* **29**, 94 (1955). Translated in Soviet Physics, *JETP* **2**, 73 (1956).
33. I. E. Dzyaloshinskii, E. M. Lifshitz and L. P. Pitaevskii, *Advances in Physics* **10**, 165 (1959).

34. H. Jehle, W. C. Parke and A. Salyers, in *Electronic Aspects of Biochemistry* (Academic Press, New York, 1964), p. 313.
35. H. Jehle, *Ann. N.Y. Acad. Sci.* **158**, 240 (1969).
36. V. A. Parsegian and D. Gingell, Am. Chem. Socy. Symposium on Recent Advances in Adhesion, Sept. 1971, Washington, D.C., to be published in *Recent Advnces in Adhesion*, ed. L-H. Lee (Gordon & Breach Science Publishers, N.Y. & London, 1972).
37. D. Gingell and V. A. Parsegian, *J. Theor. Biol.*, in press.
38. D. Gingell and V. A. Parsegian, to be published.
39. D. Gingell, in *Membrane Metabolism and Ion Transport* Ed. E. E. Bittar, (John Wiley & Sons Inc., New York, 1971), Vol. 3, Ch. 11.
40. N. Kamiya, in *Primitive Motile Systems in Cell Biology* Eds. R. A. Allen, & N. Kamiya, (Academic Press, New York, 1964).
41. D. W. James and J. F. Taylor, *Exptl. Cell Res.* **54**, 107 (1969).
42. D. R. Coman, *Cancer Res.* **4**, 625 (1944).
43. D. R. Coman, *Cancer Res.* **21**, 1436 (1961).
44. L. Berwick and D. R. Coman, *Cancer Res.* **22**, 982 (1962).
45. D. E. Brooks, *et al. J. Cell. Physiol.* **69**, 155 (1967).
46. E. W. Merrill, *et al. Circulation Res.* **18**, 437 (1966).
47. S. Roth, E. J. McGuire and S. Roseman, *J. Cell Biol.* **51**, 536. (1971).
48. S. B. Oppenheimer, *et al. Proc. Natl. Acad. Sci. U.S.A.* **63**, 1395 (1969).
49. H. Beug, *et al. Exptl. Cell Res.* **63**, 147 (1970).
50. E. Margoliash, *et al. Biochem. Biophys. Res. Commun.* **20**, 383 (1965).
51. J. E. Richmond, R. M. Glaeser and P. Todd, *Exptl. Cell Res.* **52**, 43 (1968).
52. T. Humphreys, *Exptl. Cell Res.* **40**, 539 (1965).
53. J. E. Lilien, *Devel. Biol.* **17**, 657 (1968).
54. S. B. Oppenheimer and T. Humphreys, *Nature* **232**, 125 (1971).
55. P. N. Sawyer and S. Srinivasan, Report prepared for the Artificial Heart Program, National Institutes of Health. Number RD598 U56 68–75 (1969).
56. M. C. Musolf, *et al. Trans. American Soc. Artif. Int. Organs* **15**, 18 (1969).
57. M. Costello, *et al. Trans. American Soc. Artif. Int. Organs* **16**, 1 (1971).
58. J. V. Maloney, *et al. Surgery* **66**, 275 (1969).
59. D. J. Lyman, *et al. Trans. American Soc. Artif. Int. Organs* **14**, 250 (1968).
60. K. B. Bischoff, *J. Biomed. Mater. Res.* **2**, 81 (1968).
61. F. B. Michaelis and L. Kalgarno, *Exptl. Cell Res.* **65**, 43 (1971).
62. L. Holland, *The Properties of Glass Surfaces* (John Wiley & Sons Inc., New York, 1964).
63. C. Rappaport and C. B. Bishop, *Exptl. Cell Res.* **20**, 580 (1960).
64. C. Rappaport, J. P. Poole and H. P. Rappaport, *Exptl. Cell Res.* **20**, 465 (1960).
65. M. D. Rosenberg, in *Cellular Control Mechanisms and Cancer* Ed. P. Emmelot, and O. Muhlbock, (Elsevier, Amsterdam, 1964), p. 146.
66. M. D. Rosenberg, *Protoplasm* **63**, 168 (1967).
67. S. Nordling, *Acta Path. Scand. Suppl.* **192**, 1 (1967).
68. R. E. Baier and R. C. Dutton, *J. Biomed. Mater. Res.* **3**, 191 (1969).
69. D. Gingell, *J. Theor. Biol.* **19**, 340 (1968).
70. L. Wolpert and D. Gingell, *Symp. Soc. Exp. Biol.* **22**, 169 (1968).
71. L. Wolpert and D. Gingell, in *Ciba Symposium on Homeostatic Regulators*, (J. A. Churchill Ltd., London, 1969), p. 241.