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Van der Waals Interactions between Cell Surfaces

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Summary. Van der Waals energies of interaction between model cell surfaces are calculated for various distances of separation, layer thicknesses and compositions of cell surfaces and intercellular media. In these calculations the cell peripheries are considered to consist of two layers: (1) A phospholipid-cholesterol-protein plasma membrane and (2) a surface coat, which consists of protein, sugar and water. The required Van der Waals parameters of sugars, phospholipids and cholesterol are derived from refractive indices of their solutions in the visible and ultraviolet regions. Polarizabilities and Van der Waals parameters of these substances are determined and shown to be almost independent of concentration of solutions. Resulting isotropic polarizabilities differ by less than five percent from values obtained by the addition of bond polarizabilities.

The magnitude of Van der Waals interactions between cell surfaces has been found to vary with composition according to the following sequence: water < phospholipid < cholesterol, protein < sugar. A decrease in the concentration of a given substance in the cell surface at the expense of a corresponding increase in the concentration of a substance preceding it in this sequence lowers the magnitude of attractive interactions, whereas a similar change in the extracellular medium would have an opposite effect.

A consideration of experimentally found variations in composition of cell surfaces results in calculated values of Hamaker's coefficients between 8×10^{-15} ergs and 6×10^{-14} ergs at 50 Å distance of separation, which corresponds to free energies per unit area of 210–1600 kT/μ^2.

Van der Waals forces between cell surfaces may be involved in a variety of phenomena such as cell contact and adhesion, metastasis and fusion. The main purposes of the present work are to provide an estimate for the magnitude of long range Van der Waals interactions in biological systems, based on detailed information about the dispersion equation parameters characterizing the substances composing cell surfaces and extra- and intra-cellular solutions, and to determine how changes in chemical composition at the cell periphery will affect intercellular forces. The calculations will illustrate for various geometries the variation of attractive interactions between cellular surfaces with the composition

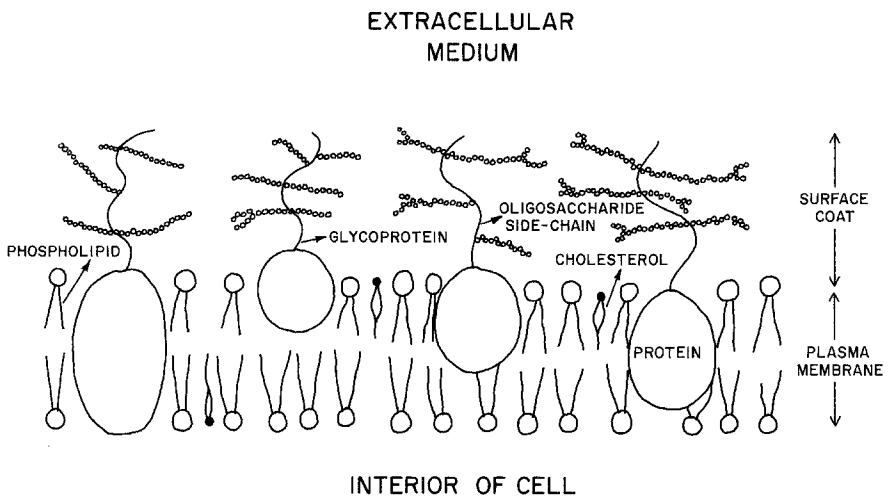

Fig. 1. The model for the cell periphery. The distance d in Eqs. (1), (8), and (9) refers to the width of extracellular medium between external boundaries of surface coats of adjacent cells

of extra- and intra-cellular solutions and cell surfaces, which will include solutions and mixtures of water, sugars, phospholipids, cholesterol and a protein. The question of specificity of Van der Waals interactions in cellular systems will also be discussed. The calculations employ a recently developed simple procedure which is based on the macroscopic approach of Lifshitz (1955). At the same time this procedure stresses the points of agreement and departure from the microscopic treatment of London (1937)–Hamaker (1937).

In order to perform these calculations we have first determined the Van der Waals parameters of several sugars, phospholipids and cholesterol from refractive indices of their solutions in the visible and ultraviolet regions. A part of the work involved measurements of refractive indices at wavelengths between 2500 and 9000 Å. In the process of this study we have also re-examined the degree of independence of the Van der Waals parameters of the molecular environment, viz., concentration of solution.

II. The Model for the Cell Periphery

In the model used here the cell periphery was considered to include a phospholipid-cholesterol-protein membrane and a surface coat containing sugars, proteins, glycoproteins and water (*see* Fig. 1). The glyco-

proteins and polysaccharides in the surface coat were approximated by simple sugars and proteins. Both the protoplasmic interior of the cell and the extracellular fluid between the cells were approximated as a dilute mixture of protein in water.

The effect of varying the amount of phospholipid, cholesterol and protein in the plasma membrane and of varying the amount of sugar, protein and water in the surface coat was studied by calculating the values of Van der Waals interaction energies for layers of different compositions, between planar sections of the cell surfaces (*see* Fig. 1).

The compositions used were based on recent biochemical studies of cell surfaces. For ghosts prepared from human erythrocytes, Hughes (1973) reports that the dry components are 50% protein, 42–43% lipid, and 7–8% carbohydrates. Values given for the dry components of the cell surfaces of rat liver cells are 39.3% lipids, 56.5% protein, and 4.2% carbohydrates (Dowben, 1971). Values given for the composition of plasma membranes of bovine erythrocytes in Ambrose and Easty (1970) are 60–70% protein, about 10% cholesterol, 19–24% phospholipids, and less than 3% other lipids. Values given for the amount of water in the cell surface are 30 to 50% (Cereijido & Rotunno, 1970). We have considered a range of compositions and layer thicknesses which covers the above reported results.

III. Theory and Method of Calculations

For a geometry of two semi-infinite slabs of materials 1 and 1′ separated by a thin film of substance 0, where all substances are dielectrics, the free energy of interaction per unit area, G, is given by Hamaker (1937) as

$$G = -A/(12\pi d^2), \tag{1}$$

in which d is the film thickness and A has been called the Hamaker coefficient and according to Hamaker (1937) is given by

$$A = \pi^2 (N_1 N_{1'} \lambda_{11'} + N_0^2 \lambda_{00} - N_1 N_0 \lambda_{10} - N_{1'} N_0 \lambda_{1'0}), \tag{2}$$

in which N_i is the number of molecules per unit volume in medium i, and λ_{ik} is the dispersion constant of London (1937),

$$\lambda_{ik} = \tfrac{3}{2} \hbar \alpha_i \alpha_k \omega_i \omega_k / (\omega_i + \omega_k), \tag{3}$$

in which $\hbar$ is Planck's constant divided by 2π and α and ω are polarizabilities and characteristic frequencies of absorption of substances i and

k. The definition of λ_{ik} in Eq. (3) corresponds to an employment of one term dispersion equation. With the definition

$$A_{ik} \equiv A_{i,k} = \pi^2 N_i N_k \lambda_{ik} \tag{4}$$

it follows that

$$A = A_{1,1'} + A_{0,0} - A_{1,0} - A_{1',0}. \tag{5}$$

A convenient relation, which uses quantities directly obtainable from the dispersion equation of the refractive index is:

$$A_{ik} = \tfrac{27}{32} \hbar B_{ik} \tag{6}$$

(Nir, 1974. *See* there also an extension for a many-term dispersion equation).

$$B_{ik} = C_i C_k \omega_i \omega_k / (\omega_i + \omega_k), \tag{7}$$

in which $C_i = 4\pi N_i \alpha_i / 3$ is the dispersion equation coefficient. Eqs. (2)–(7) consider only two body dispersion interactions and do not take into account retardation effects. An expression which takes into account many-body interactions, orientation effects and retardation effects was given by Lifshitz (1955) who developed a macroscopic theory of Van der Waals interactions, and by Dzyaloshinskii, Lifshitz, and Pitaevskii (1961). Following the method of Van Kampen, Nijboer and Schram (1968), which was further developed by Ninham, Parsegian and Weiss (1970), and by Langbein (1970), Parsegian and Ninham (1973) provide expressions for a multilayer planar system. Nir (1975) showed that the London (1937)-Hamaker (1937) approach yields values of dispersion interactions which almost coincide with those of the Lifshitz approach, and by supplementing them with orientation effects obtained a simple expression which gives essentially the same results as the Lifshitz approach:

$$A = A_{\text{disp}}(d) + \tfrac{3}{4} k T \frac{(\varepsilon_1 - \varepsilon_0)(\varepsilon_{1'} - \varepsilon_0)}{(\varepsilon_1 + \varepsilon_0)(\varepsilon_{1'} + \varepsilon_0)}, \tag{8}$$

in which ε_i is the dielectric constant of medium i, k is Boltzmann's constant and T is the absolute temperature. For short separations between bodies, $d \gtrsim 50$ Å, $A(\text{disp})$ (d) coincides with the expression given by Eqs. (2)–(7). When retardation effects are included, the magnitude of $A(\text{disp})$ is reduced. An expression which takes into account retardation effects is given by Van Silfhout (1966) who quotes Overbeek. Nir (1975) showed that this expression agrees closely with that of Dzyaloshinskii *et al.* (1961). The employment of Eq. (8) makes the calculation of Van der Waals interaction energies with the Lifshitz theory an easy task for many geometries of biological interest. For the case of the multilayer

planar system we extend and modify the approach of Vold (1961) who considered multishell spheres. Let us rewrite Eqs. (1) and (2) in the form

$$G=-A(1,1)H(d_{11}) \tag{9}$$

where

$$A(1,1)=A_{1,1}-A_{1,0}-A_{0,1}+A_{0,0}$$

and

$$H(d_{11})=\frac{1}{12\pi d^2}.$$

The symbol $A_{1,1}$ does not imply that the left hand slab consists of the same material as the right hand one, since the indices (1,1) merely indicate that we consider the interaction between the first layer to the left of the film (or medium) with the first layer to its right. For the medium, or film, in between slabs, we use the index 0. The extension to the multilayer planar case is

$$G=-\sum_{i=1}^{N_L}\sum_{j=1}^{N_R}A(i,j)H(d_{ij}) \tag{10}$$

where

$$A(i,j)=A_{i,j}-A_{i,j-1}-A_{i-1,j}+A_{i-1,j-1}+\tfrac{3}{4}kT\frac{(\varepsilon_i-\varepsilon_{i-1})(\varepsilon_j-\varepsilon_{j-1})}{(\varepsilon_i+\varepsilon_{i-1})(\varepsilon_j+\varepsilon_{j-1})}, \tag{11}$$

in which N_L and N_R denote the number of left and right layers, respectively, and d_{ij} is the distance between a layer i to the left and a layer j to the right. For instance, when $i=1$ and $j=1$, $d_{ij}=d$ and Eq. (11) is one of the same form as Eq. (8). Retardation effects have also been included in the values of the $A_{i,j}$ (*see*, Nir, 1975). The programs based on Eqs. (10) and (11) yield essentially the same results as those obtained with the employment of another set of our programs which are based on the more complicated expressions given by Parsegian and Ninham (1973). However, we have also employed Eqs. (9) and (10) for more complicated geometries, e.g., multishell spheres (*see* also Vincent, 1973). The conclusions drawn in this work apply equally well for such geometries (Nir, 1976).

IV. Determination of Van der Waals Parameters from Refractive Index Measurements

The methods of extraction of Van der Waals parameters [i.e., W_i and C_i, *see* Eqs. (3) and (7)] from refractive indices of pure liquids

are described in Gregory (1969), Nir, Rein and Weiss (1972) and Nir, Adams and Rein (1973, 1974). Our method of extraction of parameters from refractive indices of solutions is described in Andersen, Painter and Nir (1974). A few further developments and a tabulation of the real and imaginary values of refractive indices will be given elsewhere (Andersen, Painter & Nir, *in preparation*). Parameters for liquid water have already been given in Nir *et al.* (1973) and those of bovine albumin in Andersen *et al.* (1974).

We performed measurements of refractive indices of solutions for an extensive group of sugars, several phospholipids, and cholesterol at wavelengths between 2500 Å and 9000 Å. At ultraviolet wavelengths, values of refractive indices were determined from critical angle measurements made with a semicylinder-cell reflectance instrument [Painter (1968), Painter, Hamm, Arakawa & Birkhoff (1968), Painter, Birkhoff & Arakawa (1969), Andersen *et al.* (1974)]. In addition, a few measurements in the visible region were performed with a precision Abbé refractometer. Water was used as the solvent for the sugar solutions and chloroform for the cholesterol and phospholipid solutions. The dimyristoyl lecithin was synthesized in the laboratory of Dr. D. Papahadjopoulos according to the procedure described in Papahadjopoulos, Jacobson, Nir and Isac (1973) and in Papahadjopoulos and Miller (1967). Crystalline, synthetic dipalmitoyl lecithin, crystalline, synthetic sialic acid (N-acetyl neuraminic acid), and bovine phosphatidylserine were obtained from Sigma Chemical Co. Anhydrous D-glucose, L-fucose and D-galactose were used.

The results for C_i, W_i and α_i are listed in Table 1 and reflect average values for several concentrations of solutions and several procedures. The uncertainty given indicates an upper bound on the range of values. It should be stressed that the reflectance data are much less accurate than those determined with the Abbé refractometer but they have been useful in terms of supplementing the determination of W_i, which is less reliable if only visible light data are available, due to the small sensitivity of the dispersion equation in the visible part of the spectrum to values of W_i.

The results indicate that the parameters turn out to be relatively independent of concentration. The variations in concentration of solution lead to variations of up to 2.5% in the C_i coefficients and polarizabilities. It is of interest that the polarizabilities we obtain agree within 5% with those obtained by an addition of bond polarizabilities (*see* Denbigh, 1940, and Le Fevre, 1965).

Table 1. Van der Waals parameters of some cell surface components[a]

Component	Concentration of solute	Effective frequency (in units of 10^{16} rad/sec)	Coefficient, C, of solute	Electronic polarizability of solute (in units of 10^{-23} cm^3)
glucose[b]	24%, 15%, 12%	2.3 ±0.2	0.332±0.001	1.47±0.01
galactose	26%, 11%	2.1 ±0.3	0.330±0.003	1.44±0.01
fucose	30%	2.1 ±0.3	0.317±0.003	1.37±0.01
n-acetylgalactosamine[b]	18%	2.1 ±0.1	0.324±0.002	1.90±0.01
n-acetylglucosamine[b]	24%, 16%, 10%	2.1 ±0.1	0.326±0.001	1.91±0.01
sucrose	24%, 19%	2.3 ±0.15	0.326±0.002	2.78±0.02
sialic acid[b]	9%	2.0 ±0.1	0.293±0.001	2.48±0.01
cholesterol	20%, 19%	2.05±0.2	0.304±0.006	4.52±0.09
dimyristoyllecithin[b]	15%	2.0 ±0.4	0.277±0.003	7.56±0.08
dipalmitoyllecithin	20%, 15%, 10%	1.95±0.25	0.275±0.002	8.11±0.06
phosphatidylserine[b]	4%, 16%	2.0 ±0.2	0.280±0.005	8.66±0.15

[a] Values were calculated at 25 °C. Parameters for the 24% sucrose solution were obtained from data given in Krivacic and Urry (1971). All other parameters were obtained from our data.

[b] Measurements were made with precision Abbé refractometer alone.

The uncertainty in the characteristic frequencies is relatively larger. As follows from Eqs. (3) and (7), the values of Van der Waals interactions are more sensitive to C_i than to W_i. However, in calculations of Van der Waals energies we have also considered various values of W_i in the indicated range.

The polarizabilities of the different sugars vary by almost a factor of 2 (*see* Table 1). However, as was shown in section *III*, C_i and W_i are the only parameters which determine the magnitudes of long range Van der Waals interactions. Hence it is of interest that these parameters do not vary significantly within the group of sugars or within the group of phospholipids. The consideration of orientation effects requires a knowledge of the dielectric constants of the various layers, which are mixtures of substances. A program based on the Onsager (1936) equation for mixtures has been written (Nir, 1973) to provide their dielectric constants from a knowledge of molecular dipole moments and polarizabilities. Approximate values for the dielectric constants of mixtures can also be obtained from those of the components. The dielectric constant of water is well known (Weast, 1973). Dielectric constants of phospholipids varying between 2 and 10 or above are given by Hanai, Haydon

Table 2. Van der Waals attractive interaction energies between cell surfaces

Composition and dielectric constants of various layers[a]	Distances (Å)	A (dispersion) (in units of 10^{-14} ergs)[c]	A (orientation) (in units of 10^{-14} ergs)	A (total) (in units of 10^{-14} ergs)	G (in units of $10^{10}\,kT$ per cm^2)
A. Effect of distance and surface coat thickness					
Case 1					
Surface coat: 0.200 galactose, 0.200 water, 0.600 albumin, $\varepsilon=5.00$	Surface coat thickness = 0				
	$d=50$	3.0	1.4	4.4	11.7
Plasma membrane: 0.100 cholesterol, 0.500 dipalmitoyl-lecithin, 0.400 albumin, $\varepsilon=5.00$	$d=100$	1.6	0.9	2.5	1.7
	$d=200$	0.6	0.4	1.0	0.2
Case 2					
Composition same as in case 1	Surface coat thickness = 25 Å				
	$d=50$	3.4	1.8	5.2	13.8
	$d=100$	2.2	1.2	3.4	2.2
	$d=200$	1.0	0.6	1.6	0.3
Case 3					
Composition same as in case 1. Results for surface coat thicknesses of 50 Å and 100 Å are close to those presented in this case	Surface coat thickness = 75 Å				
	$d=50$	3.8	2.0	5.8	15.5
	$d=100$	2.8	1.6	4.4	3.0
	$d=200$	1.5	1.0	2.5	0.4
B. Effect of composition					
Case 4					
Surface coat[b]: 0.200 galactose, 0.500 water, 0.300 albumin, $\varepsilon=5.00$	$d=50$	1.7	2.0	3.7	9.9
	$d=100$	1.3	1.6	2.9	1.9
Plasma membrane: 0.080 cholesterol, 0.220 dipalmitoyl-lecithin, 0.700 albumin, $\varepsilon=5.00$	$d=200$	0.8	1.0	1.8	0.3
Case 5					
Surface coat[b]: 0.100 galactose, 0.200 water, 0.700 albumin, $\varepsilon=5.00$	$d=50$	3.4	2.0	5.4	14.5
	$d=100$	2.5	1.6	4.1	2.7
Plasma membrane: 0.100 cholesterol, 0.300 dipalmitoyl-lecithin, 0.600 albumin, $\varepsilon=5.00$	$d=200$	1.4	1.0	2.4	0.4

Case 6					
Same as in case 5 except for composition of surface coat which is 0.300 galactose, 0.200 water, 0.500 albumin	$d=50$	4.4	2.0	6.4	17.2
	$d=100$	3.1	1.6	4.7	3.1
	$d=200$	1.7	1.0	2.7	0.5
Case 7					
Surface coat[b]: 0.100 galactose, 0.500 water, 0.400 albumin, $\varepsilon=5.00$	$d=50$	1.4	2.0	3.4	9.1
	$d=100$	1.1	1.6	2.7	1.8
Plasma membrane: 0.100 cholesterol, 0.300 dipalmitoyl-lecithin, 0.600 albumin, $\varepsilon=5.00$	$d=200$	0.7	1.0	1.7	0.3
Case 8					
Surface coat[b]: 0.100 galactose, 0.600 water, 0.300 albumin, $\varepsilon=80.00$	$d=50$	0.89	0.011	0.90	2.39
	$d=100$	0.75	0.021	0.77	0.51
Plasma membrane: 0.100 cholesterol, 0.400 dipalmitoyl-lecithin, 0.500 albumin, $\varepsilon=20.00$	$d=200$	0.47	0.026	0.50	0.08
Case 9					
same as case 8 except ε of surface coat is 20.00, ε of plasma membrane is 10.00	$d=50$	0.9	1.1	2.0	5.3
	$d=100$	0.7	1.0	1.7	1.1
	$d=200$	0.5	0.7	1.2	0.2
Case 10					
Same as in case 8 except for composition of plasma membrane which is 0.250 cholesterol, 0.250 dipalmitoyl-lecithin, 0.500 albumin	$d=50$	0.91	0.011	0.92	2.44
	$d=100$	0.78	0.021	0.80	0.53
	$d=200$	0.50	0.026	0.52	0.09

[a] The composition of the protoplasmic interior and extracellular fluid is considered to be 0.100 albumin and 0.900 water. The value of ε is considered to be 80.00 for these mixtures. The membrane thickness is considered to be 50 Å. The coefficient and effective frequency used for albumin were 0.295 and 1.92×10^{16}. Values used for the coefficients and effective frequencies were taken from Table 1 for galactose, cholesterol and dipalmitoyl lecithin, from Andersen, Painter and Nir (1974) for bovine serum albumin and from Nir, Adams and Rein (1973) for water. "d" is thickness of intercellular space, i.e., between external extensions of surface coats.

[b] Surface coat thickness is considered to be 75 Å.

[c] Retardation effects are considered in this calculation.

Table 2 (continued)

Composition and dielectric constants of various layers[a]	Distances (Å)	A (dispersion) (in units of 10^{-14} ergs)[c]	A (orientation) (in units of 10^{-14} ergs)	A (total) (in units of 10^{-14} ergs)	G (in units of $10^{10}\ kT$ per cm^2)
Case 11					
Cell 1: composition same as case 7	$d=$ 50	2.2	2.0	4.2	11.4
Cell 2: composition same as case 5	$d=100$	1.7	1.6	3.3	2.2
	$d=200$	0.9	1.0	1.9	0.3
Case 12					
Cell and glass; cell: same as case 4	$d=$ 50	4.4	2.1	6.4	17.2
Glass parameters: C=0.33, w=2×10^{16} rad/sec, $\varepsilon=7$	$d=100$	3.5	1.8	5.3	3.5
	$d=200$	2.2	1.4	3.6	0.6
Case 13					
Cell and coated glass; cell: same as case 4. Thickness of	$d=$ 50	2.2	0.4	2.6	7.0
coating layer is 50 Å; composition 50% protein,	$d=100$	2.1	0.7	2.8	1.9
50% water, $\varepsilon=80$	$d=200$	1.6	0.8	2.4	0.4

and Taylor (1964), Ohki (1970), Schwan, Takashima, Miyamoto and Stoeckenius (1970), Fettiplace, Andrews and Haydon (1971), and Redwood, Takashima, Schwan and Thompson (1972). There is uncertainty regarding the permanent dipole of a protein molecule, which leads to widely varying estimates for the dielectric constant of a protein containing medium. Since measurements of the dielectric constants of layers of the compositions considered are complicated because of polarization effects, we have used a range of values. Fortunately, in most cases, dispersion interactions give the major contribution to Van der Waals interactions at short distances of separation. This was shown by Nir (1974, 1975) and is illustrated in Table 2 which gives separately the contribution of dispersion effects and orientation effects, where the latter depend on the chosen set of ε values.

V. Results and Discussion

Cell separations of 50 Å, 100 Å and 200 Å were considered. Calculations were done for plasma membrane thicknesses of 50 and 100 Å and surface coat thicknesses of 0, 25, 50, 75 and 100 Å.

In Table 2 we illustrate the results of calculations of Van der Waals interactions, representing 2000 variations in composition, dielectric constants, surface coat and plasma membrane thicknesses, and cell separations.

Galactose was used as a representative sugar for the saccharides in the surface coat and dipalmitoyl lecithin as a representative phospholipid in the membrane. This choice was based on the results in section *IV* that the parameters varied only slightly within the sugar and phospholipid groups. For cholesterol and phospholipids higher values of W_i were also considered.

Bovine serum albumin was used as a representative protein. Although this may not be a typical protein in the surface coat, it was the closest substance to a typical protein for which data were available. Our preliminary results indicate that the parameters of another protein, actin, are close to those of albumin.

Our results showed that an increase of phospholipids at the expense of protein or cholesterol in the plasma membrane slightly decreased the attractive Van der Waals forces. A reduction in water content or an increase in sugar content of the surface coat layer increased the

magnitude of interaction energy. These results may be summarized by writing down a sequence of substances in the form: water < phospholipid < cholesterol, protein < sugar. The meaning of this sequence in terms of the magnitude of attractive interactions between cellular or subcellular systems is that a replacement in the cell surface of an amount of a substance in the above sequence by an amount of a substance to its left in the sequence would cause a decrease in the magnitude of attractive interactions and vice versa. Similar changes in the extracellular medium would lead to reversed effects. This means that stripping a surface coat of a cell of a large amount of its glycoproteins would cause a significant reduction in the magnitude of attractive interactions. If glycoproteins, proteins or sugars which do not stick to the cell surface are added to the extracellular medium the effect would be a significant reduction in the magnitude of attractive interactions between cell surfaces. A removal of phospholipids from the cell membrane to the extracellular medium would have a minor effect on long range attractive interactions since the reduction due to their introduction into the medium is compensated by the slight enhancement due to their removal from the plasma membrane.

A consideration of the Van der Waals parameters of the alkanes reported by Ingram (1974) indicates that they have C values varying from 0.21 for C_5H_{12} to 0.25 for $C_{15}H_{32}$, with an approximate increment of 0.002 per additional carbon in the chain. Hence the alkanes would occupy a position between water and phospholipids in the above sequence. The results for alkanes are consistent with our results that varying the number of CH_2 groups of the phospholipids considered here would still leave their C values within the range used in Table 2.

It has to be emphasized that the values of A in Eq. (1) are also functions of the distance of separation and of the layer thicknesses. An increase in d will cause a reduction in A values because of retardation effects. The results in Table 2 show reduction by a factor of about 2 to 3 when d is increased from 50 to 200 Å. It is to be noted that contrary to the case of two semi-infinite, planar surfaces separated by a medium, the contribution of orientation effects to A values shows some distance dependence for this multilayer case. Similarly there is some distance dependence in the contribution of dispersion interactions to A; even at distances where retardation effects are small. In our system the presence of the surface coat generally enhances the magnitude of A so that we have a reduction with distance, d, of the contribution of dispersion interactions to A. An increase in plasma membrane thickness resulted in

a slight increase in the magnitude of attractive interactions. For instance, when values of 100 Å were used instead of 50 Å a 10 to 20% increase was obtained.

The effect of surface coat thickness on the magnitude of A is more complicated because of a combination of several factors. Without consideration of retardation effects the magnitude of attractive interactions would generally tend to increase with an increase in surface coat thickness (*see* cases 1 to 3 in Table 2). The explanation is that the composition of the surface coat is such that it yields larger attractive interaction than a layer of the composition of the membrane. A reduction in the water content of the surface coat would enhance this effect, whereas an increase in its water content would lead to an opposite effect. In certain cases a maximum in A values was found for a certain value of surface coat thickness.

A consideration of possible variations in composition and thickness of the layers gave a wide range of A values. For $d=50$ Å, A values range from 8×10^{-15} ergs to 6×10^{-14} ergs corresponding to values of G of 210 to 1600 kT/μ^2 ($1\ kT=4\times10^{-14}$ ergs). For $d=200$ Å the range of A values is 4×10^{-15} ergs to 2×10^{-14} ergs. The values given in other studies (Curtis, 1967; Parsegian & Gingell, 1973; and Weiss *et al.*, 1975) are included in this range.

In flocculation experiments a mean value of $1.6\pm1.0\times10^{-14}$ ergs was found for A (Wilkins, Ottewill & Bangham, 1962). The relatively low value of 0.5×10^{-14} which was suggested by Weiss (1968) from an analysis of cell detachment experiments, falls within the range of our values for A.

It may be of interest to compare the magnitude of Van der Waals forces in cellular systems with that of mechanical forces acting on cells. For the purpose of such a comparison consider the magnitude of gravitational forces, i.e., $mg\Delta\rho$, where $\Delta\rho$ is the difference in density between the cell and the medium. For a cell of mass $m=2\times10^{-9}$ gm and $\Delta\rho=0.1$ gm/cm^3 the gravitational force is 2×10^{-7} dynes. The expression for Van der Waals force is obtained from Eq. (10) where $H(d_{ij})$ is now $1/(6\pi d_{ij}{}^3)$. For brevity we did not provide values for the forces, but for the cases given in Table 2 the attractive forces vary between 4 and 30×10^{-5} dynes/μ^2 at a distance of separation of 50 Å. These values are more than two orders of magnitude larger than the ordinary gravitational forces, but at $d=300$ Å these forces are comparable and even for $d=20$–50 Å, strong centrifugal forces can be applied to overcome the effect of Van der Waals forces.

We have also included in the calculations a system of a cell and a substrate. In case 12 in Table 2 the results for a special glass substrate are given. The relatively high value of A for cell-substrate is typical of most inorganic solids. In case 13 we consider a more realistic case of glass coated with a protein layer. Here the magnitude of the interaction falls within the range of values for the cell-cell system.

The effect of variations in the extracellular medium or serum and its constituents on cellular adhesion is complicated, because the medium affects many other parameters of cell activity. Ballard and Tomkins (1970) showed that hepatoma cells require serum for adhesion. Srere and Milam (1974) find that baby hamster kidney (BHK) cells adhere better in the absence of serum. Curtis (1973) gives an additional extensive list of references for cases where sera or sera factors promoted adhesion and for cases where they diminished adhesion. Our calculations (*see* Table 2 and previous paragraphs) indicate that an inert serum is expected to diminish the adhesion process, and the effect may be emphasized in the case of trypsinized cells. In fact, when serum which does not absorb to cells becomes very concentrated the attractive Van der Waals interactions between homotypic cells can drop down to zero, and for heterotypic cells they can even become negative. Of course, if the serum contains molecules which can adsorb to cellular surfaces then it will promote adhesion by the formation of microextensions which can help to overcome the repulsive electrostatic barrier which always exists due to the fact that cellular surfaces [of vertebrate at least (Weiss, 1968)] carry net negative charges.

We believe that our calculations can provide an explanation for the contradictory effects of neuraminidase on cellular adhesion [*see* Curtis (1973) for review]. Neuraminidase treatment of cells results in lowering of their surface charge density by removal of sialic acid, which comprises a large fraction of the sugar content of the surface coat. A decrease in the magnitude of the negative charge density would lower the magnitude of the electrostatic repulsive interactions. In contrast, as we have discussed, a removal of sugar from the surface coat results in a significant reduction in the magnitude of attractive interactions between two cells or a cell and a substrate, particularly when water replaces the sugar. Therefore it can be expected that both the attractive Van der Waals interactions and the repulsive interactions will be reduced in magnitude, not necessarily in the same fashion, so that their sum can be either reduced or enhanced. We hope to be able to estimate these changes when the accompanying changes in the cell surface potential and the amount of material removed from the cell surface are known.

This wide range of values of A can lead to phenomena of specificity of interaction (Jehle, 1969) due to Van der Waals forces before contact is established. We also illustrate in Table 2 that in the general case of two types of cells, a and b, the relation $^1/_2\ (Gaa+Gbb)<Gab$ is satisfied, in which Gaa or Gab refers to the attractive free energy per unit area of apposed cells, a or b, at the same distance of separation. A comparison (cases 5, 7 and 11 in Table 2) shows that at $d=50$ Å, $Gaa=-1450$, $Gbb=-910$, and $Gab=-1140$ in units of kT/μ^2. Note that $Gaa<Gab<Gbb$ and that Gab is larger than $^1/_2\ (Gaa+Gbb)$, which is $-1180\ kT/\mu^2$. This result means that attractive long range Van der Waals interactions exhibit specificity of long range recognition during the initial stages of cell-cell contact and adhesion, at distances of 300 Å or less. At such distances the system of four cells a, a, b, b will have a reduction in an excess of kT per unit area of μ^2 in the segregated state of pairs aa, bb compared with the mixed state ab, ab [*see also* Good (1972)]. It should be noted that electrostatic (repulsive) long range interactions do not satisfy the relation $^1/_2\ (Gaa+Gbb)<Gab$ so that long range specificity of recognition in cell-cell interactions can be attributed only to the Van der Waals interactions (as was stressed by Parsegian and Gingell, 1973).

However, our calculations can point out unambiguously cases where specificity of intercellular interactions cannot be explained on the basis of long range interactions.

In Table 1 we have presented parameters for the four sugars, fucose, galactose, *n*-acetyl glucosamine and *n*-acetyl galactosamine which are thought to be present in varying amounts in blood groups A, B and O to determine their specificities (Kabat, 1956; Winzler, 1970). Our calculations indicate that there is only a slight difference in long range Van der Waals interactions when different blood group sugars are present in the surface coat. Thus we believe that the specificity of interactions related to the A, B and O groups cannot be explained in terms of long range interactions. In consideration of contact and adhesion the long-range Van der Waals effect must be combined with other effects, e.g., short range interactions, motility, transfer of information through diffusion, etc. It should be emphasized that, even in cases where adhesion between cell surfaces is very sensitive to the presence of specific molecules on the corresponding surfaces, the prerequisite for the short range (a few Å) molecular recognition to occur is the ability of cell surfaces to approach each other closely enough. The process of cell contact and cell adhesion involves an initial stage during which the cells are still far apart. Van der Waals forces can be most important in bringing

cells to a distance of separation at which short range forces can begin to act. Thus, at distances of several hundred Angstroms and less (down to a few Å) long range interactions can be of primary importance in determining the magnitude of the attractive forces pulling parts of apposing cells together.

In this study we have quantitated the magnitude of long range Van der Waals interactions with a special emphasis on its dependence on gross compositions of cell surfaces and media. This information when applied to analysis of differences in contact and adhesion behavior of cellular systems under varying conditions can in some cases explain and predict these differences on the basis of long range interactions, or, in other cases, may be useful in stimulating consideration of other mechanisms.

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