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DETERMINATION OF CELL/MEDIUM INTERFACIAL TENSIONS FROM CONTACT ANGLES IN AQUEOUS POLYMER SYSTEMS

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Summary

The contact angles on cell layers of a series of polymeric droplets from aqueous two-phase systems of dextran and poly(ethylene glycol) have been used to determine the critical or limiting interfacial tension for spreading on the cell layers. Test droplets of the denser dextran-rich phase were formed in the lighter poly(ethylene glycol)-rich phase. The interfacial tensions, γ, between the phases were determined with the pendant drop method, and a linear relationship was found between $\gamma^{-1/2}$ and the cosine of the angle the droplets made with the cell layers (Good-Girifalco plot). We were thus able to determine the limiting or critical interfacial tension, γ_c, for spreading on the cell layers. The value of γ_c is a measure of the interfacial energy of the cell/bathing medium interface. Values of γ_c obtained by this method include the following: 0.65 and 0.84 $\mu N \cdot m^{-1}$ for human erythrocytes and neutrophils, respectively; 0.93 $\mu N \cdot m^{-1}$ for porcine pulmonary macrophages; 0.75—3.60 $\mu N \cdot m^{-1}$ for various transformed murine lymphoid cell lines, and 2.53 $\mu N \cdot m^{-1}$ for Balb/c murine spleen lymphocytes. Exposure to various agents has differing effects on γ_c. Concanavalin A reduces γ_c, and bacterial lipopolysaccharide increases γ_c of murine spleen lymphocytes. The calcium ionophore, A23187, increases γ_c of both porcine pulmonary macrophages and murine spleen lymphocytes. This new method provides a quantitative approach to the cell surface energy and hydrophobicity which are thought to play an important role in membrane-mediated phenomena and in cell adhesion.

Abbreviations: A-MuLV: Abelson murine leukemia virus; θ, contact angle of a liquid drop on a plane surface; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

Introduction

The adhesion of cells to other cells or to foreign substances, phagocytosis, pinocytosis, fusion and agglutination are all events involving the cell surface. Spontaneous interactions between the surfaces of cells, subcellular components, or foreign substances will only occur if the interactions are accompanied by a negative free energy change, but few measurements of the surface energies of living cells are available.

The importance of interfacial free energies in interactions at the cell membrane, such as phagocytosis [1], erythrocyte aggregation [2] and the coalescence of cells with oil droplets, were recognized by early workers, and during the 1930's ingenious mechanical methods were developed to estimate 'tensions at cell surfaces' [3]. A variety of cells were deformed in the centrifuge microscope [4] or by the use of gold microbeams [5] to yield estimates of the cell surface tensions which lie within the range 0.6—3 $mN \cdot m^{-1}$. The results of these experiments played an important role in the development of the Davson-Danielli model of the cell membrane [6] by demonstrating that cells contained substances which were capable of greatly reducing the tension at an oil/water interface. In general outline, this view is still held today, but the mechanical estimates of interfacial tension on which it was based are not completely satisfactory since they measure both elastic and surface tensions, and cannot distinguish between elastic forces of the cell surface and those of the interior.

The importance of the interfacial tension for the cell/medium interface in cell-cell adhesion has been emphasized repeatedly [3,37,38] but methods to obtain direct measurements have been lacking.

Van Oss et al. [7] approached the problem of phagocytic engulfment from the point of view of surface energy. They estimated differences in surface energy from contact angles of saline droplets on air-dried films of cells. Their method offered a simple way to test the virulence of isolated bacteria: only those bacteria with a larger contact angle (more hydrophobic) than those of neutrophils were readily engulfed by the neutrophils, while bacteria with smaller contact angles than those of neutrophils resisted engulfment. These measurements have provided considerable insight into the role of surface hydrophobicity in processes such as phagocytosis and microbial adhesion, but it is difficult to extrapolate them to the in vivo state, since cells normally exist in an aqueous environment.

Because of the singular importance of water in biological function in general [8], and in both the kinetics and energetics of cell surface interactions [9,10], we have developed a method of measuring the interfacial tensions of living cells in an aqueous environment which approximates their in vivo situation. The approach is based on the contact angle method for a liquid/liquid/solid system previously described [11, 12]. Initially, we used hydrophobic test fluids of varying polarity and measured the contact angles made by droplets of the fluids on layers of cells immersed in Ringer's solution [13,14]. This approach yielded very low values (approx. 1 $\mu N \cdot m^{-1}$) for the cell/water interfacial tensions, but the use of hydrophobic test fluids is not without problems; the high interfacial tension between the test fluid droplet and the bathing fluid reduces the sensitivity of the method, and the possibility of pharmacological

interactions between the test fluid and the underlying cells cannot be ignored. The low interfacial tension of the cell/medium interface requires a careful choice of the liquids to be used for contact angles measurements: the interfacial tension between the bathing fluid and the test droplet should be of the same order of magnitude as the interfacial tension of the cell/bathing fluid interface. Immiscible pure liquid pairs (e.g., combinations of hydrocarbons, fluorocarbons and silicones with water) not only have high interfacial tensions, they also tend to respond primarily to the apolar component of the interfacial tension at the cell surface.

We have partially circumvented these problems by using two-phase test systems composed of aqueous solutions of the polymers, dextran and poly(ethylene glycol). Water-soluble polymers such as poly(ethylene glycol) are confined to the extracellular phase in a variety of cell types [15] and unlike hydrophobic polymers, are capable of sustaining cellular homeostasis even under conditions which would otherwise be lethal [16]. Having these desirable attributes, and having extremely low interfacial tensions, the immiscible phases of aqueous polymer mixtures provide a new, sensitive, nonperturbing and quantitative method of measuring interfacial tensions at cell surfaces. In the present study we show that the interfacial tensions of the cell polymer solution range from 10 to 0.6 $\mu N \cdot m^{-1}$, depending on the cell type, and that the interfacial tensions of the cell/medium interface are modified by concanavalin A, bacterial lipopolysaccharide and the calcium ionophore, A23187.

Materials and Methods

Aqueous two-phase systems

The method is based on a series of aqueous two-phase systems of dextran (mol. wt. 500 000, Pharmacia) and poly(ethylene glycol) (mol wt. 20 000 or 6000, Fisher or Sigma). We made a first series of six two-phase systems using dextran (mol wt. 500 000) and poly(ethylene glycol) (mol.wt. 20 000) of varied concentrations. For a second series of six two-phase systems, we used dextran (mol. wt. 500 000) and poly(ethylene glycol) (mol. wt. 6000). The polymers were dissolved in Ringer's solution and put in 500-ml glass cylinders for the phase separation. The highest concentration for the first and the second series was 5% (w/w), the lowest concentration was 3% (w/w) for the first series, and 4% (w/w) for the second series. According to Albertsson [17], the time taken for the phases to separate for a dextran/poly(ethylene glycol) system varies from 15 to 60 min. We always allowed 6—10 h for this equilibration process.

Both the lighter poly(ethylene glycol)-rich phases and the denser dextran-rich phases were then removed from the glass cylinders and stored separately in a refrigerator. The compositions of the different phases were calculated from determinations of the water content and the concentration of one of the polymers [17]; the concentration of the other polymer was then obtained by subtraction. Fig. 1 is the phase diagram for the system (molecular weight in parentheses) dextran (500 000)/poly(ethylene glycol) (20 000) obtained by drawing the curve through the points describing the compositions of the two phases of each system. The 'tie line' is obtained by drawing the line through point P that

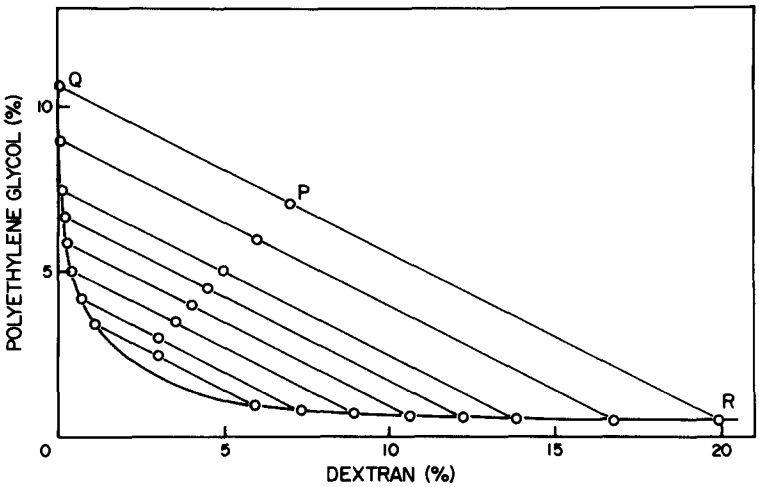

Fig. 1. Phase diagram of aqueous mixtures of dextran (mol wt. 500 000) and poly(ethylene glycol) (mol. wt. 20 000). Point P represents the composition of the total system (percent of dextran and poly(ethylene glycol) per total weight mixture), while Q gives the polymer concentrations (percent of dextran and poly-(ethylene glycol) per weight of the poly(ethylene glycol)-rich phase) and R gives the polymer concentrations in the dextran-rich phase. All mixtures having a total composition on the tie line QR separate into two phases having the compositions of Q and R.

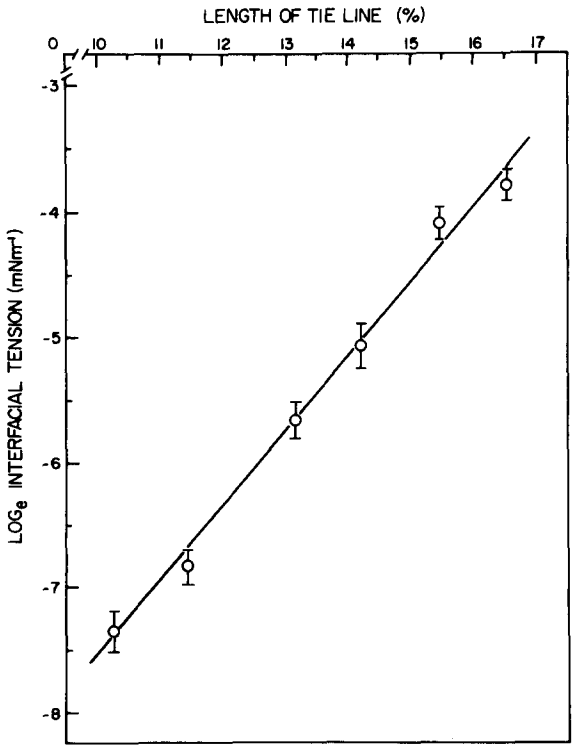

Fig. 2. Interfacial tensions between the phases formed by mixtures of dextran (500 000) and poly-(ethylene glycol) (20 000) plotted vs. the length of the tie lines (see Table I and Fig. 1). Error bars indicate ±2 S.E.

represents the composition of the total system (percent of dextran and poly-(ethylene glycol) per total weight mixture). The composition of the top and the bottom phases is represented by points Q and R.

Fig. 2 demonstrates the linear relationship for the system dextran (500 000)/ poly(ethylene glycol) (20 000) obtained by plotting the logarithm of the interfacial tensions between the phases against the lengths of the tie lines. A similar result was obtained by Ryden and Albertsson [18] using a system composed of dextran (500 000) and poly(ethylene glycol) (6000).

Interfacial tensions

We used the pendant drop method [19] to determine the low interfacial tensions (1.0—0.0001 mN · m^{-1}) between the dextran-rich phase and the poly-(ethylene glycol)-rich phase of the two-phase systems. All measurements were carried out at room temperature. Because of the low interfacial tensions, glass micropipettes of a tip diameter of approx. 10—50 μm had to be pulled. We found that only micropipettes with flat and smooth tips were suitable for our measurements. The micropipettes were mounted onto a micromanipulator and connected with Teflon tubing to a 1 ml microsyringe. Droplets of the denser, dextran-rich phase were then formed inside the lighter, poly(ethylene glycol)-rich phase in a glass cuvette with optically flat faces. The drops were then photographed by means of a Nikon Optiphot microscope equipped with a differential interference attachment. The magnification was 250×. The original pictures were projected onto a screen, where the necessary parameters for the determination of the interfacial tensions were measured. For each system, the interfacial tension was measured six to ten times. We found the results to be reproducible with a relatively low standard error of the mean (approx. 6%). The measured values for the system dextran (500 000) and poly(ethylene glycol) (20 000) are given in Table I. For the system consisting of dextran (500 000) and poly(ethylene glycol) (6000), the interfacial tensions given by Ryden and Albertsson [18] were used. We confirmed their values (to within the standard error of the mean of less than 7%) by using the pendant drop

TABLE I

INTERFACIAL TENSIONS BETWEEN THE TWO PHASES OF AQUEOUS MIXTURES OF DEXTRAN (500 000) AND POLY(ETHYLENE GLYCOL) (20 000)

The composition is expressed as percent of dextran and poly(ethylene glycol) per total weight of the mixture. Numbers in parentheses indicate the number of measurements with the pendant drop method.

Composition		Interfacial tension	
Dextran (500 000) (%, w/w)	Poly(ethylene glycol) (20 000) (%, w/w)	(μN · m^{-1})	(±S.E.)
5.0	5.0	22.8 ± 1.42	(9)
4.5	4.5	17.0 ± 1.11	(10)
4.3	4.3	6.41 ± 0.561	(6)
4.0	4.0	3.47 ± 0.260	(8)
3.5	3.5	1.11 ± 0.0721	(6)
3.0	3.0	0.643 ± 0.0562	(8)

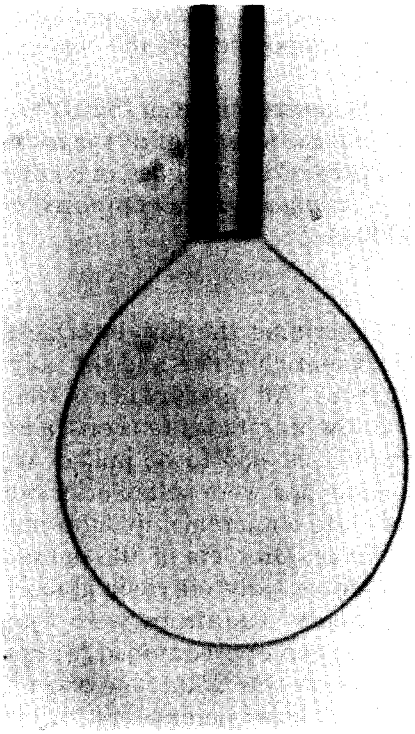

Fig. 3. Pendant drop for the two-phase system consisting of dextran (500 000) and poly(ethylene glycol) (20 000), 5% each per total weight mixture. For this particular system, the interfacial tension was approx. 0.023 mN · m^{-1}. The outside diameter of the micropipette is 36 μm. The photograph was taken with a Nikon Optiphot differential interference microscope (250X).

method. Fig. 3 shows a photograph of a pendant drop for the two-phase system dextran (500 000)/poly(ethylene glycol) (20 000), 5% each (w/w). For this particular system the interfacial tension was approx. 0.023 mN · m^{-1}.

Preparation of cells

Monolayers of isolated cells. Monolayers were prepared by gentle filtration of cells from suspensions onto hydrated cellulose acetate membranes with a pore diameter of 0.45 μm (Millipore Corp.). Cells were counted in a hemocytometer, and the final cell density on the filter was adjusted to 2—5 · 10^3 cells/μm^2.

Human red blood cells. These cells were separated by centrifugation from freshly drawn citrated whole blood.

Human leucocytes. Human leucocytes were separated from citrated whole blood by gradient centrifugation in 2% dextran (70 000). The erythrocytes were lysed with 0.8% NH_4Cl and the leucocyte pellet was washed three times and resuspended in phosphate-buffered Ringer's solution.

Porcine pulmonary macrophages. The pulmonary macrophages were prepared by saline lavage of isolated porcine lungs, followed by centrifugation at 400 × *g* for 7 min.

Cell lines. Cell lines used were as follows: lymphocytic lines, donated by B. Weimann, were: K(mouse, Balb/c, A-MuLV transformed) [20], BM18-4 (Balb/c, bone marrow, A-MuLV transformed in vitro) [21], and ABLS-1 Balb/c, A-MuLV transformed in vivo) [22]. The lymphocytic lines were maintained in Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY). P815 (mouse, DBA/2, a monocytic mastocytoma line commonly used as a target in killer cell assays) [23], was donated by M. Julius. and maintained in RPMI 1640 (Gibco). Both media contained 10% fetal calf serum, and all cultures were incubated in 5% CO_2/95% air at 37°C.

Murine spleen cells. Spleen cells were obtained either from Balb/c or C57BI/nu 6—8-week-old animals obtained from the Institut für Biologisch-Medizinische Forschung AG, Füllinsdorf, Switzerland. Spleen cells were obtained by macerating spleens through a stainless-steel screen and removing erythrocytes by lysis with 0.8% NH_4Cl. Cells were washed three times and resuspended in RPMI 1640 containing 50 μM 2-mercaptoethanol, 20 μM Hepes and 5% fetal calf serum. Cell viability was routinely assessed by the trypan blue exclusion method. All experiments reported were performed on preparations of which the viability was greater than 90% as judged by this method.

The critical or limiting surface tension for spreading

We have adapted the method of critical surface tension for spreading for aqueous two-phase systems. The concept of the critical surface tension for spreading was originally introduced by Zisman [24] to characterize the surface free energies of solids. The critical surface tension, γ_c, is determined by measuring contact angles with the substrate in question for each of a series of liquid droplets of known surface tension and plotting the cosine of these angles against the surface tension of the droplet. The critical surface tension for the substrate is then defined as the intercept of the line of best fit through the data points with the axis for $\cos\theta = 1$. Physically, γ_c separates liquids which do not form finite contact angles with the substrate (liquids that spread spontaneously) from those which do form finite contact angles and do not spread.

In our studies the substrate was a layer of cells immersed in a bathing fluid consisting of the light, poly(ethylene glycol)-rich phase while the dense, dextran-rich phase provided the test fluid droplet. Thus, the interfacial tensions between the polymeric phases replace the surface tensions of the original concept of Zisman [24].

In general, the phase combinations with the highest concentration of polymers produced the highest interfacial tensions; for example, in the system dextran (500 000)/poly(ethylene glycol) (20 000), 5% (w/w) each, the interfacial tension between the two phases was approx. 0.02 $mN \cdot m^{-1}$, while for the lowest concentration, 3% (w/w) each, the interfacial tension was 0.6 $\mu N \cdot m^{-1}$. We placed test fluid droplets of diameters between 0.1 and 1.0 mm onto layers of cells that had been immersed in the bathing fluid. The viscosity of the polymer solutions varies with polymer concentration, so the kinetics of droplet spreading varied between the different test solutions. With the lowest polymer concentrations, constant contact angles were achieved within 1 min; with the highest concentrations 10—15 min were required for equilibration. After this initial period, no further change in contact angle was observed for 2 h or more.

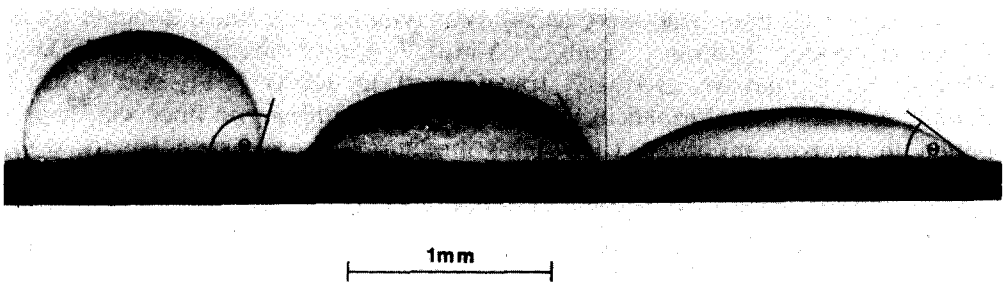

Fig. 4. Droplets from the heavy phases of three two-phase systems of dextran (500 000) and poly-(ethylene glycol) (20 000) on layers of human red cells immersed into the light phases. The concentrations (w/w) of each of the polymers and the contact angles were: 5.0%, 106° (left); 3.7%, 61° (middle); 3.3%, 38° (right). The photographs were taken with a Nikon stereomicroscope, and by using polarized light.

The right and the left contact angles of each drop were measured either directly with a protractor eyepiece or determined from photographs. The precision of these measurements was ±1°. The angle was taken as the final advancing contact angle.

The change in droplet-cell contact angle with changing polymer concentra-

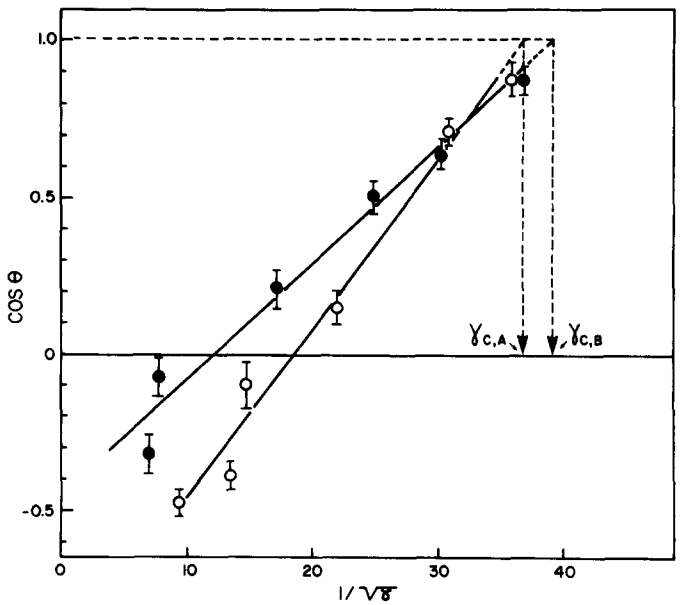

Fig. 5. Good-Girifalco plots for the determination of γ_c, the critical interfacial tension for spreading on human red cells. Each point is obtained in a different two-phase system composed of dextran (500 000) and poly(ethylene glycol) (6000) (series A), ○———○; or dextran (500 000) and poly(ethylene glycol) (20 000) (series B), ●———●. The lowest point to the left is for the system with the highest concentration (5% dextran, 5% poly(ethylene glycol) while the highest point to the right if for the most dilute mixture, 3.5% for each of the two polymers, for series B. For the same series, when we used the two-phase system of 3.0% dextran and poly(ethylene glycol), the test fluid droplets spread completely on the cell layers, indicating a zero-degree contact angle. Thus, $\gamma_{c,B}$ is determined with respect to a system between 3.0 and 3.5% of the two polymers, In this example, $\gamma_{c,A}$ is 0.74 $\mu N \cdot m^{-1}$ while $\gamma_{c,B}$ is 0.65 $\mu N \cdot m^{-1}$. The two values are within the error range of approx. 8%. Error bars indicate ±2S.E.

tion provides the basis for the determination of γ_c. For example, on human erythrocytes, the contact angle decreased from approx. 110° to 40° with decreasing polymer concentrations (Fig. 4).

The measured interfacial tensions, γ, and the corresponding contact angles, θ, enabled us to plot γ vs. $\cos\theta$ according to Zisman [24]. However, the relationship between γ and $\cos\theta$ for our system was far from linear.

Good [25,26] recommended determining the critical interfacial tension, γ_c, by an extrapolation of a plot of $\gamma^{-1/2}$ vs. $\cos\theta$ (Good-Girifalco plot) which should give a much more linear relationship than the original approach of γ vs. $\cos\theta$ (according to Zisman [24]). The expectation that a plot of $\gamma^{-1/2}$ vs. $\cos\theta$ should yield a straight line follows from the analysis of Good's interaction parameter [27].

Fig. 5 demonstrates that for erythrocytes the series of two-phase systems of dextran (500 000)/poly(ethylene glycol) (6000) and of dextran (500 000)/poly(ethylene glycol) (20 000) produced a linear relationship of $\gamma^{-1/2}$ vs. $\cos\theta$. Correlation coefficients obtained for Good-Girifalco plots for various cell types with either series of two-phase systems ranged from 0.999 to 0.850. For a particular cell type and a particular batch thereof, we made 10—20 contact angle measurements for each of our polymer combinations (Table I). The measurements from a particular cell batch were treated as a sample. An analysis of variance at the 5% level of significance showed that all of the samples were taken from the same population, and hence the results from at least four different batches of a particular cell type were pooled. Therefore, each point on the plot was obtained from 40—100 contact angle measurements. The standard error of the mean was between 2 and 5%.

Results

Good-Girifalco plots of data obtained from human erythrocytes with dextran (500 000)/poly(ethylene glycol) (6000) (plot A) and with dextran (500 000)/poly(ethylene glycol) (20 000) (plot B) are given in Fig. 5. From plot A, we found the critical interfacial tension for spreading $\gamma_{c,A} = 0.74\ \mu N \cdot m^{-1}$, while the value obtained from plot B was $\gamma_{c,B} = 0.65\ \mu N \cdot m^{-1}$. Confidence intervals of 95% were calculated for each of the extrapolated values $\gamma_{c,A}$ and $\gamma_{c,B}$ at $\cos\theta = 1$. These confidence intervals overlapped which indicates a low probability of the γ_c values being different for group A and B. Thus, we concluded that both polymer systems used gave equivalent results for the limiting interfacial tension γ_c.

Fig. 6 demonstrates the change in contact angle following exposure of porcine pulmonary macrophages to the calcium ionophore, A23187. The droplets were from the heavy phase of a two-phase system dextran (500 000), and poly(ethylene glycol) (20 000), 5% (w/w) each. The contact angle on normal macrophages was 130° (left), while after exposure to 1 μM A23187, the contact angle was 92°. The Good-Girifalco plots (Fig. 7) showed a large change in γ_c of macrophages exposed to the calcium ionophore ($\gamma_{c,B} = 1.5\ \mu N \cdot m^{-1}$) compared to normal macrophages ($\gamma_{c,A} = 0.93\ \mu N \cdot m^{-1}$).

Table II summarizes our findings for nine cell types under various conditions. Concanavalin A, bacterial lipopolysaccharide and calcium ionophore

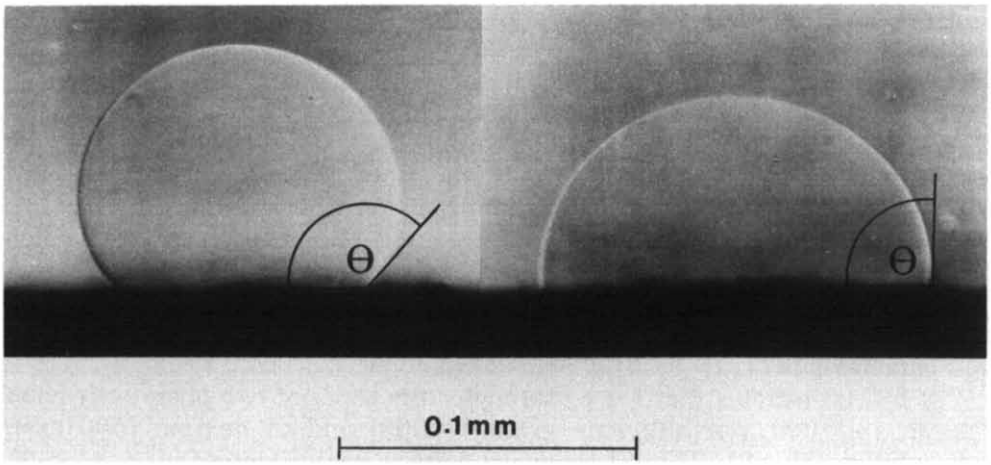

Fig. 6. Change of contact angle (θ) due to exposure of porcine pulmonary macrophages to 1 μM calcium ionophore A23187. On normal macrophages, θ was 130° (left), while on macrophages exposed to the calcium ionophore, θ was 92° (right). Both droplets were from the heavy phase of a two-phase system of dextran (500 000) and poly(ethylene glycol) (20 000), 5% (w/w) each. The photographs were taken with a Nikon Optiphot differential interference microscope (200X).

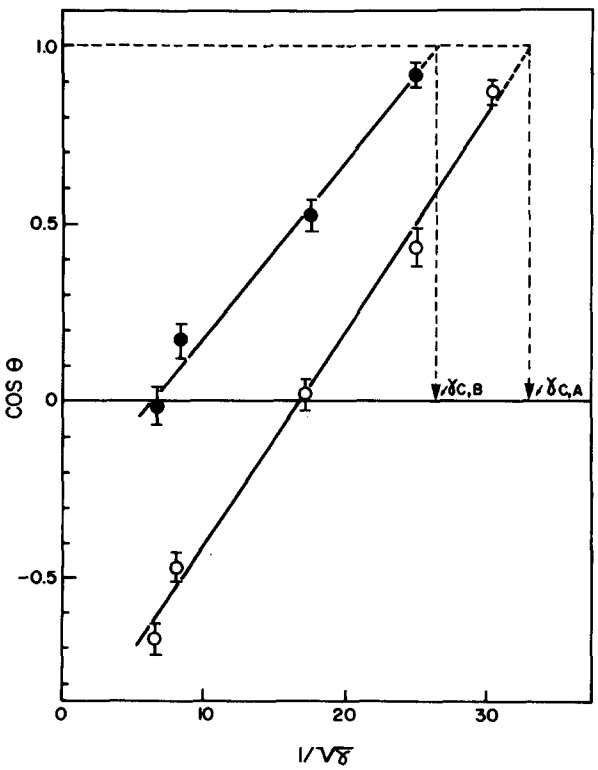

Fig. 7. (○———○) Good-Girifalco plot of normal porcine pulmonary macrophages, with a critical interfacial tension of $\gamma_{c,A} = 0.93\ \mu N \cdot m^{-1}$ and of macrophages (●———●) that had been exposed to 1 μM calcium ionophore A23187, $\gamma_{c,B} = 1.5\ \mu N \cdot m^{-1}$. Error bars indicate ±2S.E.

TABLE II

CRITICAL INTERFACIAL TENSIONS OF γ_c OF MAMMALIAN CELLS WITH RESPECT TO DEXTRAN/POLY(ETHYLENE GLYCOL) TWO-PHASE SYSTEMS

The critical interfacial tensions (γ_c) for spreading were determined from a Good-Girifalco plot (line of best fit), utilizing four to six two-phase mixtures with 40—100 contact angle (advancing) measurements for each. The estimated error of γ_c is ±2—6% (S.E.), as determined from the graphs of $\gamma^{-1/2}$ vs. cos θ.

Phase systems and cells			γ_c ($\mu N \cdot m^{-1}$)
I	Dextran (500000)/poly(ethylene glycol) (20 000)		
	(A)	Human: erythrocytes	0.65
		Human: neutrophils	0.84
	(B)	Porcine pulmonary macrophages:	0.93
		normal + A23187 (1 μM)	1.46
II	Dextran (500 000)/poly(ethylene glycol) (6000)		
	(A)	Human: erythrocytes	0.74
	(B)	Murine spleen cells	
		(1) Balb/c: normal	2.53
		Balb/c: + concanavalin A (5 μg/ml)	2.22
		Balb/c: + lipopolysaccharide (25 μg/ml)	3.47
		Balb/c: + A23187 (1 μM)	4.40
		(2) c57BI/nu: normal	10.11
		c57BI/nu: + concavanavalin A (5 μg/ml)	6.40
		c57BI/nu: + lipopolysaccharide (25 μg/ml)	9.88
	(C)	Murine cell lines	
		(1) K	1.10
		(2) BM18-4	3.33
		(3) ABLS-1	3.60
		(4) P815	0.75

A23187 are all considered to produce changes in the cell surface following binding [28—30]. The widespread use of these agents as tools in cell biology prompted us to determine whether binding of these agents resulted in a change in the surface energy of the cell. Balb/c spleen cells, approx. 50% T and 50% B lymphocytes, show a decrease in γ_c following exposure to 5 μg/ml concanavalin A. Increases in γ_c occur following exposure to lipopolysaccharide. C57BI/nu spleen cells, consisting only of B lymphocytes, also show a decrease in γ_c with concanavalin A, but show little alteration with lipopolysaccharide. Thus, although concanavalin A specifically stimulate T cells and lipopolysaccharide specifically stimulates B cells, over a short time period (2 h) this does not correlate with alterations in cell surface free energy, as determined using γ_c. Long-term study of γ_c of stimulated lymphocytes may enable the detection of cell membrane phospholipid alterations which are known to result from stimulation with mitogenic lectins [31]. In both porcine pulmonary macrophages and Balb/c spleen cells, calcium ionophore A23187 (1 μM) causes an increase in the limiting interfacial tension γ_c of more than 20% compared to normal macrophages.

Discussion

Because we used a series of two-phase systems, each having different phase composition, the limiting or critical interfacial tension for spreading is esti-

mated in each case relative to a slightly different bathing fluid. The polymer concentration in the bathing fluid for a particular γ_c value can be estimated from the tie line length corresponding to the interfacial tension γ_c (Figs. 1 and 2): for example, for red cells at the critical interfacial tension, the bathing fluid is a poly(ethylene glycol)-rich phase of approx. 5.4% poly(ethylene glycol) (20 000), while in the case of neutrophils the critical interfacial tension is relative to a bathing fluid containing 5.6% poly(ethylene glycol) (20 000). Murine spleen cells and lymphocytic cell lines demonstrate the highest interfacial tensions. Again, these values are relative in a particular poly(ethylene glycol)-rich phase of varied poly(ethylene glycol) concentrations.

The two-phase polymer mixture corresponding to γ_c provides two liquid phases having special interfacial tensions at the cell surface: the interfacial tension between the light, poly(ethylene glycol)-rich phase and the cell is identical to γ_c, which in turn is identical to the interfacial tension between the two liquid phases; the interfacial tension between the heavy, dextran-rich phase and the cell is a minimum and may be equal to zero. The critical interfacial tension, γ_c, for the substrate is closely related to and is a measure of the substrate/bathing fluid interfacial free energy [24].

In general, the interfacial tension between the test fluid droplets and the substrate approaches a minimum as the interfacial tension between the two liquid phases approaches γ_c [32]. This minimum value may be equal to zero depending on whether the intermolecular forces across the test fluid/substrate interface are of the same type as those within the test liquid and the substrate [33]. The interfacial tension between two identical polymer mixtures is zero, and as differences in composition increase one would expect an increase in the interfacial tension. Zero interfacial tension between unlike polymer mixtures would indicate a great similarity in the polar and apolar contributions to the interfacial tension. For example, for red cells at γ_c, the molecular properties of the red cell surface must closely match those of a dextran-rich phase containing approx. 9.5% dextran (500 000), while the neutrophil surface at γ_c would be similar to a dextran-rich phase containing 10.1% dextran (500 000). This follows from an extrapolation from Figs. 1 and 2. Measurements of lactic dehydrogenase levels in the supernatant (unpublished data) confirmed that 1 μM A23187 is non-cytotoxic to pulmonary alveolar macrophages. It has recently been shown that this concentration of the ionophore causes virtually complete loss of pyroantimonate-stainable calcium from the cell surface [34]. A consideration of the thermodynamics of divalent ion exchange [10] predicts that calcium displacement should increase the cell surface hydrophilicity, thus lowering the free energy of the cell/water interface. Dextran is more hydrophilic than poly(ethylene glycol) [17], so the decreased cell/dextran interfacial tension indicated in Fig. 6 may well reflect alterations in cell surface hydrophilicity following calcium displacement by A23187.

In attempting to relate these measurements to the biological milieu it is necessary to consider the relationship between γ(cell/polymer solution) and γ(cell/extracellular fluid) of cells in their native state. No measurement can be made without some perturbation by the measuring process. The question that arisis is thus: to what extent does the presence of the polymers in the test solutions alter the interfacial energy at the cell surface? Clearly, at the polymer

concentrations employed in this study, no gross alterations is to be expected, since the aqueous two-phase partition method has been extensively validated as a gentle procedure for the isolation of viable cells [17]. However, more subtle effects arising from polymer adsorption at the cell/test fluid interface are quite likely, since hydrophilic polymers such as dextran [35] and poly(ethylene glycol) [15] are retained by cell surfaces.

'Adsorption' and 'spreading' reflect the same intermolecular interactions and polymers which do not adsorb to some extent are unsuitable for probing interfacial energies by measurements of wetting. However, from the arguments discussed above, it is clear that polymer adsorption must affect the interfacial energy somewhat, since adsorption can only proceed spontaneously if the adsorption process lowers the interfacial energy. Estimating the influence of polymer adsorption on the measured γ_c value thus requires a method of estimating cell surface energy which is independent of polymer adsorption.

Our earlier studies of cell surface wetting by immiscible hydrophobic test fluids [13] allow a partial resolution of this problem: despite the insensitivity of hydrophobic test fluids to the ultra-low interfacial energies reported in the present work, there is nevertheless striking agreement between γ_c measured on erythrocytes with dextran (500 000)/poly(ethylene glycol) (20 000) (0.65 $\mu N \cdot m^{-1}$) and γ(solid/liquid) estimated with isopropyl salicylate (0.95 $\mu N \cdot m^{-1}$) (Schürch, S. and McIver, D.J.L., unpublished results). Thus, it appears that polymer adsorption from the test fluids is not responsible for the ultra-low free energies at the cell surface, but rather, that these energies reflect the fact the cell surface is itself composed of high molecular weight hydrophilic polymers, the interfacial properties of which resemble those of other polymer solutions.

To workers accustomed to the high tensions observed at lipid/vapor interfaces (approx. 1—25 $mN \cdot m^{-1}$), the magnitude of the interfacial tensions reported in this study will doubtless seem very low, and the physiological importance of such weak interactions may seem uncertain. However, as pointed out by Cowell et al. [36], a particle covered by terminally anchored polymers may be regarded as a giant polymer molecule, the core of which has been replaced by the material of the particle. These workers have shown that mixtures of such particles have thermodynamic properties similar to those of the corresponding polymers in free solution. Edwards [37] has drawn attention to the similarity between polymer phase separation of the type employed in this study and the differential adhesiveness of cell types during growth and differentiation. It is also interesting to compare the present measurements with earlier estimates of the 'tensions at cell surfaces' derived from measurements of the mechanical deformation of cells [3—6]. These earlier estimates are much higher than the present measurements (approx. 1 $mN \cdot m^{-1}$ compared with 1 $\mu N \cdot m^{-1}$); the differences are reduced when comparison is made with methods in which it was sought to minimize the effects of deformation [5], suggesting that anisotropy in the response of cells to mechanical stress is the basis for the differences and that with respect to interfacial tension cells behave more like elastic solids than like liquids.

The determination of γ_c as a measure for the cell/medium interfacial free energy may provide a useful tool in studies of cell surface properties such as cell surface hydrophobicity and cell-cell adhesion [38,39]. The quantitative

methods and measurements provided here yield estimates for cell/medium interfacial free energies from which free energies of adhesion between cells of various types could be calculated. In addition, it may provide a means of discovering aqueous solutions having zero interfacial tension against a given cell type.

The present study shows that cell/medium interfacial tensions derived from contact angle measurements in aqueous polymer systems vary in a reproducible fashion between different cell types, and between the same cell types under different conditions. Ongoing investigations are aimed at elucidating the molecular basis of the measured interfacial tensions.

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