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Intercellular Adhesion: Modification by Dielectric Properties of the Medium

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Summary. The low radio frequency dielectric constant of aqueous solutions of glycine, diglycine, D-sorbitol, Dextran and Ficoll were determined. These values were used to predict the dielectric constant of Hanks-199 tissue culture medium to which various concentrations of these compounds had been added. Single cell dispersions of two chick embryonic tissues, 7-day neural retina and 5-day limb bud, were prepared in tissue culture media of varying dielectric constant. Selected cell dispersions were examined by means of particle electrophoresis and the observations of zeta potentials were interpreted as showing that no significant adsorption of added compounds in the media was occurring onto the cell membranes.

Cell suspensions in media of a range of dielectric constant were subjected to a laminar flow shear gradient in a couette viscometer, effecting aggregation of these suspensions. This method allowed a calculation of the total energy of adhesive interaction of the cells. It was shown that 5-day limb bud tissue has a much lower adhesive interaction energy than 7-day neural retina tissue. It was observed that in both tissues there was a steady increase in the adhesive interaction of the cells with increasing dielectric constant of the medium. These results are discussed in relation to the lyophobic colloid stability theory of cell adhesion.

The mechanisms involved in cell adhesion have been the subject of considerable study. The work of Moscona (1961, 1962) was thought to point to the existence of specific intercellular 'cements' which were responsible for the adhesion of given cell types. Both Steinberg (1964) and Pethica (1961) have proposed modifications of the cementing theory, implicating calcium ions to act as ion-pair or triplet bridges between carboxyl groups on the cell surface (Haydon & Seaman, 1962). One aspect of this theory is that cells are considered to be in molecular contact, with a separation of less than 20 Å between the apposing cell surfaces. Although this view has been supported by the work of Wilkins, Ottewill and Bangham (1962*a*, *b*)

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on the aggregation of leucocytes at zero point of charge, Curtis (1967), in a review of the subject, has pointed to a considerable body of evidence for the existence of two major classes of cell adhesion. It appears that although certain specific cell-to-cell adhesions may be in molecular contact, many cells seem to adhere with a separation of some 80 to 150 Å between the membranes.

From a theory proposed by Derjaguin and Landau (1941) and Verwey and Overbeek (1948) on the stability of lyophobic colloids, (DLVO theory), Curtis (1966) has suggested that cell adhesion may be the result of a balance between two opposing physico-chemical forces. The repulsive component of the interaction is a consequence of the presence of charged groups of the same sign on the two cell surfaces. This gives rise to an electrostatic force of repulsion due to the surface potential existing on each membrane. The attractive force is the London van der Waals force, which is determined by the molecular nature of the adhering surfaces and by the material present in the gap between them.

Little experimental evidence has been produced which supports or disproves either the DLVO or the cementing theories. However, it should be possible to test the DLVO theory by altering the dielectric constants of the medium in which cells are immersed, since electrostatic theory indicates that variation of potential and force is dependent upon the dielectric constant of the medium (Kruyt, 1952). Theoretical calculations of the total interaction potential energy curves for particles in which the dielectric constant of the medium has been varied show that adhesiveness can be affected by this parameter (Brooks, Millar, Seaman & Vassar, 1967; Gingell, 1971; Jones, 1972). On the other hand, the dielectric constant is not a factor generally considered in other theories of cell adhesion, although chemical interactions between cell surfaces clearly will be affected by this parameter.

The basis of the theory on the stability of lyophobic colloids is a potential energy curve composed of an electrostatic repulsive potential energy and an attractive energy. For a pair of identical spherical particles, the repulsive potential energy V_R for large k_a values is given by

$$V_R = \frac{a\varepsilon\psi^2}{2} \quad \ln(1+\exp(-kh)) \tag{1}$$

where ε is the static dielectric constant of the medium, a the radius of the particle, h the minimum distance between the surfaces of the particles, ψ the surface potential (assumed to approximate to the zeta potential) and k the Debye-Huckel reciprocal length. The equation is valid for zeta potentials < 25 mV and $a \gg h$.

The potential energy of attraction V_A is normally given by

$$V_A = \frac{-A a}{12h} \tag{2}$$

where A is the London van der Waals or Hamaker constant. The calculation of Hamaker constants has traditionally been approached on the assumption of additivity of intermolecular dispersion forces (Gregory, 1969). This method has been criticized on a number of grounds (Parsegian & Ninham, 1969) and the calculation of attractive energy using the macroscopic approach devised by Lifshitz (1956) and his co-workers (Dzyaloshinskii, Lifshitz & Pitaevskii, 1960) is now preferred. Here the expression for the attractive force is given in terms of the macroscopic dielectric susceptibilities of the interacting materials and variation of this parameter will affect the energy of attraction. This method formally requires a knowledge of the dielectric data over a wide frequency range of measurement; however, Ninham and Parsegian (1970) have performed calculations of the attractive force in several model systems using restricted dielectric data.

Complete quantitative calculations based on the Lifshitz theory are not yet possible with the complex media used in studies of cell reaggregation; nevertheless, simple predictions may be made on the nature of the energetic effects when changing the dielectric properties of these media.

Materials and Methods

Determination of Cell Adhesiveness

Use was made of a method devised by Curtis (1969) in which a monodispersed cell suspension is reaggregated in a couette viscometer. A detailed theoretical description of the method is given elsewhere (Curtis & Hocking, 1970), the essentials of the method being the measurement of the number of collisions which produce adhesions per unit time and the use of a technique which allows a calculation of the total number of collisions per unit time. The probability that a collision between two cells results in an adhesion is termed the collision efficiency and is given by (Curtis, 1969)

$$\ln \frac{N_{\infty t}}{N_{\infty 0}} = \frac{-4G\phi\alpha t}{\pi} \tag{3}$$

where $N_{\infty t}$ and $N_{\infty 0}$ are the total number of particles at time t and time zero, respectively, G is the shear rate, ϕ is the volume fraction of particles in suspension and α is the collision efficiency. If cells can be aggregated in a laminar system of known shear rate, it is possible to measure the collision efficiency directly. The couette viscometer provides such conditions of laminar flow, the experimental procedure simply being to carry out aggregations in a couette viscometer, counting the total number of particles at frequent intervals.

The viscometers used in this study were designed and constructed in the workshop of the Engineering Department of Glasgow University. The cylinders were made of EN58B stainless steel which is resistant to corrosion by saline solutions. The inner cylinder, 19 mm in diameter, is freely suspended on air bearings, while the outer cylinder, 20 mm in diameter, is rotated to give a constant rate of shear by means of an integrating motor. Speed of rotation can be varied by changes of voltage or gearbox to give shear rates from 2 sec^{-1} to well above 20 sec^{-1}, the shear rate being calculated from the angular velocity of the rotating cylinder and the measured radii of the two cylinders (Jones, 1972). The viscometers were placed in a constant temperature room some hours before use (2.5 $\pm$ 0.5 °C).

Measurement of Dielectric Constant

Several methods have been described to estimate the sample capacitance and dielectric constant from the total measured impedance. Oncley (1938) showed that at high frequencies measured capacitance tended to sample capacitance. Schwan and Maczuk (1960) showed that a log plot of apparent dielectric constant as a function of electrode separation enabled the true dielectric constant to be estimated. Where electrode polarization is negligible, Young and Grant (1968) were able to obtain the sample capacitance from a linear plot of measured capacitance against the square of measured conductance. If an electrode polarization contribution is present (as would be the case with sample solutions of high conductivity measured at low frequencies) the technique devised by Fricke and Curtis (1937) may be employed. The dielectric constant of the solution can be obtained without knowing the absolute value of the polarization impedance since this parameter is eliminated in the calculations (Schwan, 1963). Dielectric constants are calculated from the measured capacitances C_1 and C_2 corresponding to the electrode spacings d_1 and d_2 by means of the relationship (Shaw, 1942)

$$\varepsilon = [d_2^2(C_2 - C_0) - d_1^2(C_1 - C_0)]/K(d_2 - d_1) \tag{4}$$

where C_0 and K are constants for a particular dielectric measuring cell. This method was chosen as the most suitable for the present study.

To avoid major contributions from electrode polarization, measurements of capacitance were determined in the low radio frequency range, where values of dielectric constant will not be dissimilar from static values. The apparatus consisted of a signal generator, bridge, detector and measuring cell. The combined signal generator and detector was type SR268 (Wayne Kerr Co. Ltd., Surrey) covering the frequency range 100 kHz to 100 MHz. The bridge was type B201 (Wayne Kerr Co. Ltd.), a 3-terminal transformer ratio-arm admittance bridge providing readings of capacitance and conductance over the frequency range 100 kHz to 5 MHz. The measuring cell was a permittivity jig D321 (Wayne Kerr Co. Ltd.) modified to hold liquids by glueing a piece of Perspex, 25 mm deep with a 52-mm diameter hole in it, centrally onto the base plate. This jig is a 3-terminal device with a guard ring design to ensure a uniform field in the sample. All connections between units were made with grounded co-axial cable, lead lengths being kept to the absolute minimum.

All capacitance measurements were performed at the same temperature at which cell aggregation and electrophoresis were conducted, the apparatus being set up in a constant temperature room (2.5 $\pm$ 0.5 °C). Measurements could be made on solutions with specific conductivities as high as 200×10^{-4} ohm^{-1} m^{-1}. Determinations were usually carried out at solution pH 7.45, this being the value of the aggregating media in which cells were suspended.

Dielectric constant values were obtained for glycine, diglycine, Dextran (mol wt 15,000 to 20,000), D-sorbitol and Ficoll. Ficoll was obtained from Pharmacia who report an average mol wt of 400,000, D-sorbitol from Koch-Light and the other three compounds from Sigma. Solutions were made up in double glass-distilled water and brought to the desired pH with 0.01 M triethanolamine (BDH).

Preparation of Tissue Cell Suspensions

Both 7-day neural retina cells and 5-day limb bud cells from Dekalb chick embryos were used in this study. The cells were prepared following the technique of Curtis and Greaves (1965) using EDTA saline as the disaggregating medium. Cells were dispersed using a fine bore Pasteur pipette and the suspension forced through nylon cell sieves (Nitex, New York) of 10-μm mesh size to ensure that the vast majority of small clumps were removed. The cell concentration was determined by haemocytometry and appropriate dilutions made to bring the total particle concentrations of experimental and control suspensions to equality.

Solutions

Hanks saline, calcium and magnesium-free saline (CMF) and EDTA saline were made up following Curtis and Greaves (1965). The suspension media used in cell aggregation and electrophoresis were based on a 1:1 ratio of Hanks and Medium 199 (Flow Laboratories). The various compounds used to alter the dielectric constant were made up in double glass-distilled water before being added to a 10× concentrate of Medium 199; pH was then adjusted to 7.45 with sodium hydroxide. Control solutions were modified by additions of D-sorbitol and sodium chloride to give equal pH, ionic strength and osmolarity to experimental and control suspension media.

Cell Electrophoresis

The apparatus employed was a particle microelectrophoresis MkII device constructed by Rank Bros., Bottisham, Cambridge. A thin-walled cell of circular cross-section was employed with a system of blacked platinum electrodes. The stationary levels of the measuring cell were determined by a standard technique (Kruyt, 1952, p. 219) and timing of particle transits were performed with the electromagnetic clock provided which is accurate to 0.02 sec. The apparatus was set up in a constant temperature room at 2.5 ± 0.5 °C; temperature fluctuations were minimized to a maximum of ± 0.2 °C with the use of a thermostatted water bath. Cell suspensions were prepared as previously described and before use the electrophoresis cell was rinsed with distilled water and the cell and electrodes were then washed in the cell suspension. Cells were timed in alternate directions to minimize possible polarization effects at a field strength of 6.04 V/cm. Ten particles were timed in each direction for each stationary level, giving a total of 40 readings for each experiment. Transit times for the cells at this field strength fell within the range of 6 to 11 sec.

Results

Determination of Dielectric Constants

As a check on the accuracy of the measuring technique, the dielectric constants of water, methanol and ethanol were measured at 100 kHz.

Table 1. Measured values of dielectric constant

Solution		pH	Dielectric constant	Frequency (kHz)
Glycine				
0.1 M	unbuffered	6.4	89.48	500
0.1 M	buffered	7.45	89.31	500
0.5 M	unbuffered	6.2	99.02	500
0.5 M	unbuffered	6.2	99.25	100
0.5 M	buffered	7.45	98.60	500
1.0 M	unbuffered	6.1	111.5	500
1.0 M	buffered	7.45	110.7	500
Diglycine				
0.1 M	unbuffered	5.5	95.01	500
0.2 M	unbuffered	5.3	103.15	500
D-Sorbitol				
5% w/v	buffered	7.45	86.57	100
5% w/v	buffered	7.45	87.17	500
5% w/v	buffered	7.45	87.10	1000
Dextran				
2% w/v	buffered	7.45	86.22	500
5% w/v	buffered	7.45	85.60	500
5% w/v	buffered	7.45	85.20	100
5% w/v	buffered	7.45	85.50	1000
Ficoll				
1% w/v	buffered	7.45	86.70	500
5% w/v	buffered	7.45	86.08	100
5% w/v	buffered	7.45	86.09	500
5% w/v	buffered	7.45	86.09	1000

Values obtained for double glass-distilled water at 20 and 25 °C were 80.45 and 78.40, respectively. At 20 °C methanol gave a value of 33.67 while at 25 °C, 60% ethanol and 20% ethanol had dielectric constants of 48.00 and 69.40, respectively. These figures are in excellent agreement with those given in NBSC 514 (*see* Maryott & Smith, 1951), NBSC 589 (*see* Buckley & Maryott, 1958) and the CRC Handbook of Chemistry and Physics (1966). The mean value obtained for water at 2.5 °C was 87.14. Further studies at 2.5 °C on the variation in the dielectric constant of glycine with measurement frequency and electrode separation indicated that measured values would fall within 2% of the theoretical value given by Wyman and McMeekin (1933) at frequencies over 200 kHz and electrode separations of 2.5 mm and 5.0 mm. The specific conductivity of the solutions employed gave the final

limitations to the accuracy of measurement. Circuit inductance must be low to satisfy the general approximations used to derive Eq. (4) (Shaw, 1942; Mandel, 1965). To estimate this, induction was measured using a technique described by Schwan (1963) under the unfavorable conditions of high conductivity. Values for inductance fell around a mean of 0.6×10^{-6} H, well within the limits allowed.

The conductivities of physiological media are far too high to allow for a direct measurement of their dielectric properties. Determinations were therefore carried out on simple aqueous solutions of the various compounds. All these solutions, except where specifically stated, were made up to the same osmolarity and pH as the media used in the cell studies. Table 1 gives the values measured in this study. In all instances the dielectric constant of water was measured just previous to the test solution, this acting as a check on the performance of the apparatus.

Examination of Cell Dispersions

Cell dispersions were scrutinized prior to use in the various media by phase contrast microscopy. Cells in suspension in all the media except 1.0 M glycine and the corresponding control were approximately spherical in shape. In the latter two media the cells appeared very crenated. Cell diameter, irrespective of media, was reasonably constant with a value for 5-day limb bud of 6.95 ± 0.6 μm (SE) and for 7-day neural retina of 5.90 ± 0.8 μm (SE). The cell suspensions prior to use consisted of at least 90% single cells, the remainder being made up of cell doublets and triplets in an approximate ratio of 3:1. Cell viability was assessed by a plating-out technique to investigate the possibility of cell injury or death in the modified media. Cells were plated at densities of 1×10^3/ml at 25 °C. After 1 hr the proportion of cells which had settled and spread was measured. Cells which had settled were judged to be alive. Normally more than 70% of cells were judged alive by this technique, but for both 5-day limb bud and 7-day neural retina the percentage judged viable in 1.0 M glycine medium was less than 50%.

Cell Reaggregation

In all the reaggregations performed, a plot of $\ln N_{\infty,t}$ against t gave a straight-line relationship, indicating that the aggregation followed the kinetics given in Eq. (3). The values for the collision efficiency given in Table 2 for 7-day neural retina and 5-day limb bud are all based on at least four separate experiments. The shear rate chosen differs between the two cell types, a rate of 10.24 sec^{-1} being used with neural retina and

Table 2. Estimation of collision efficiency (%)

Aggregation media	Cell type			
	7-Day neural retina		5-Day limb bud	
	Control ± SE	Experiment ± SE	Control ± SE	Experiment ± SE
Hanks-199	15.41 ± 0.321	–	2.40 ± 0.142	–
0.1 M Glycine	15.26 ± 0.254	16.80 ± 0.322	2.30 ± 0.134	2.86 ± 0.202
0.5 M Glycine	14.63 ± 0.658	19.09 ± 0.522	2.35 ± 0.062	7.60 ± 0.360
1.0 M Glycine	16.24 ± 0.868	22.54 ± 0.748	1.33 ± 0.456	5.89 ± 0.543
0.1 M Diglycine	14.93 ± 0.431	18.36 ± 0.219	2.25 ± 0.219	5.075 ± 0.204
0.2 M Diglycine	14.99 ± 0.297	19.73 ± 0.309	2.47 ± 0.067	7.35 ± 0.092

Table 3. Calculation of force constants

Aggregation media	Collision efficiency (%)	Interaction parameter H	Force constant M (joules)
(a) 7-Day neural retina			
D-Sorbitol control	15.26	5.5158×10^{-7}	5.651×10^{-23}
0.1 M Glycine	16.80	7.2913×10^{-7}	7.535×10^{-23}
0.5 M Glycine	19.09	1.9338×10^{-6}	2.068×10^{-22}
1.0 M Glycine	22.54	5.4163×10^{-6}	5.948×10^{-22}
0.1 M Diglycine	18.36	1.541×10^{-6}	1.603×10^{-22}
0.2 M Diglycine	19.73	2.3572×10^{-6}	2.529×10^{-22}
(b) 5-Day limb bud			
D-Sorbitol control	2.30	8.451×10^{-10}	3.662×10^{-26}
0.1 M Glycine	2.86	1.354×10^{-9}	5.918×10^{-26}
0.5 M Glycine	7.60	2.440×10^{-8}	1.104×10^{-24}
1.0 M Glycine	5.89	9.974×10^{-9}	4.633×10^{-25}
0.1 M Diglycine	5.075	6.223×10^{-9}	2.738×10^{-25}
0.2 M Diglycine	7.35	2.1568×10^{-8}	9.792×10^{-25}

2.65 sec^{-1} for limb bud. These rates were found by trial to be the most suitable values for the cell type concerned.

From the collision efficiency values it is possible to calculate the adhesive interaction of the cells from the relationship (Curtis, 1969):

$$M = 72\pi\eta a^3 GH \tag{5}$$

where H is a function of the collision efficiency given by $H = 10^{1.178\alpha\frac{1}{2}-10.86}$, M is termed the force constant and η is the viscosity of the medium which must be taken into account. Eq. (5) assumes that the repulsive forces are negligible (Curtis & Hocking, 1970), an assumption that will lead to an underestimation of the Hamaker value. Table 3 shows values for these parameters calculated from a variety of reaggregation experiments.

Cell Electrophoresis

Calibration of the apparatus was performed using human red blood corpuscles, the recommended procedure (Seaman, 1965). The mobility of washed erythrocytes at 25 °C in 0.145 M sodium chloride solution was calculated as 1.11 ± 0.03 μm/sec/volt/cm. This is in good agreement with other figures quoted in the literature. Both 7-day neural retina and 5-day limb bud suspensions were examined under identical conditions of applied voltage (50 V) and temperature (2.5 °C). Cell mobilities were observed and zeta potentials calculated using the Helmholtz-Smoluchowski equation (Collins, 1966). The results are given in Table 4. The zeta potentials in various media were not significantly different at the 1% level using the Students *t* test when compared with their controls.

Table 4. Measurement of zeta potential

Suspension media	Mobility (μm/sec/volt/cm ± SE)		Zeta potential (mV ± SE)
(a) 7-Day neural retina			
Hanks-199	0.63 ± 0.002		−13.9 ± 0.04
0.5 M Glycine	0.61 ± 0.005		−12.5 ± 0.09
D-Sorbitol control	0.54 ± 0.002		−12.4 ± 0.03
		$p = \gg 0.1$	
0.2 M Diglycine	0.63 ± 0.001		−12.3 ± 0.10
D-Sorbitol control	0.52 ± 0.002		−12.5 ± 0.05
		$p = \gg 0.1$	
(b) 5-Day limb bud			
Hanks-199	0.65 ± 0.002		−14.4 ± 0.03
0.5 M Glycine	0.64 ± 0.003		−13.2 ± 0.05
D-Sorbitol control	0.58 ± 0.001		−13.3 ± 0.03
		$p = \gg 0.1$	
0.2 M Diglycine	0.66 ± 0.002		−13.2 ± 0.09
D-Sorbitol control	0.56 ± 0.013		−13.5 ± 0.32
		$p = \gg 0.1$	

Zeta potentials for control and experimental values compared by Students *t* test. Probabilities given in the Table.

Zeta potentials were corrected for viscosity and dielectric constant.

Discussion

The figures obtained for the dielectric constants generally agree well with the findings of other workers. The dielectric increment for glycine falls within the range of 23.1 to 24.5, compared with a value of 23.6 given by Wyman and McMeekin (1933). Similarly with diglycine, dielectric

increment values vary from 80.1 to 80.75 compared with values of 84.4 and 80.0 quoted by Young and Grant (1968). Solutions of D-sorbitol gave values of dielectric constant similar to that of water. Though no values for this compound could be found after a search of the literature, this result could be predicted from a knowledge of its structure. Sorbitol, having an optical rotation of $(\alpha)\ ^{20}D - 2.0$, may in solution tend to reduce the dielectric constant below that of water, much in the same manner as sucrose (Pethica, 1961), though perhaps not so effectively. Study of the figures given for Dextran and Ficoll in the Results show that, broadly, the two compounds exhibit similar behavior with respect to concentration and measurement frequency. Both compounds in solution show a somewhat decreased dielectric constant compared to water. The general conclusion for solutions of Dextran, Ficoll and D-sorbitol is that at frequencies at or below 1 MHz the dielectric constant remains very similar to that of water. Except for glycine and diglycine solutions no increase in the dielectric constant above that of water was recorded.

Pollack, Hager, Reckel, Toren and Singher (1965) have also studied the dielectric properties of Dextran and Ficoll solutions, and their findings do not agree with those given here. They report dielectric increments for Ficoll and Dextran of 14.70 and 40.45, respectively. Thus a 5% w/v Ficoll solution would give a dielectric constant value of 152, while 5% Dextran would have a value of 280.75. No simple explanation can be given for the discrepancies between the two sets of results. Brooks *et al.* (1967) originally lent support to the value of the dielectric increment for Dextran obtained by Pollack *et al.* (1965). They were able to show that correction of the electrophoretic mobility values of erythrocytes by the dielectric constant of Dextran obtained by Pollack *et al.* (1965) gave mobility values almost independent of the Dextran concentration. However, in later communications (Brooks, 1971; Brooks & Seaman, 1972) it was reported that the mobility effects were not due to any solution properties of Dextran, but possibly to counter-ion exclusion by the adsorbed polymer. More recently, Brooks and Seaman (1973) have measured the dielectric constant of Dextran solutions and found that these are indistinguishable from that of water. They also refer to a study performed by Allgén and Roswall (1954) which arrived at the same conclusion that Dextran had no significant effect on the dielectric constant of water.

Cell electrophoretic studies were performed to test for the effects of glycine and diglycine, together with the D-sorbitol control media, on both neural retina and limb bud cell suspensions. The results show that the calculated zeta potentials in both the experimental and control media are

very similar and agree well with the values quoted in the literature for these cell types measured in simple saline solutions (Collins, 1966; Curtis, 1967). It is therefore concluded that with none of the compounds used in cell aggregation studies does any significant adsorption to the cell surface occur, since on adsorption large changes in zeta potential would be predicted (Abramson, Moyer & Gorin, 1942). If zeta potential is equated with surface potential, as is conventional though not strictly accurate, it can be said that the presence of D-sorbitol, glycine and diglycine in the media do not significantly change the surface potential of the cell membranes. The relationship between zeta potential and surface charge density reveals that as the dielectric constant is increased the zeta potential is decreased. The range of dielectric constant covered here is not wide enough to allow for an appraisal of this effect and it can probably be safely assumed that changes in the dielectric constant have no effect on the zeta potential of the cells.

The values for collision efficiency, determined from the aggregation of limb bud and neural retina suspensions can be used in two ways; as a means of calculating an absolute value for the force constant M, or as a way of comparing the rates of aggregation when these are carried out under similar conditions. The figures given in Table 2 demonstrate three overall factors in the aggregation of the cell suspensions. Except in the case of aggregations involving 1.0 M glycine and its control, the controls themselves give very similar results for collision efficiency within the two cell types. This argues in favor of the test system being an excellent method for obtaining reproducible results. A second point concerns the difference in the aggregation capabilities between the two cell types. The limb bud tissue in Hanks-199 medium at a shear rate of 2.65 sec^{-1} gave a collision efficiency of 2.4%, while neural retina at a shear rate of 10.24 sec^{-1} gave a value of 15.41%. When shear rates are taken into account this difference in collision efficiency would be even more pronounced. Neural retina cells aggregated at a shear rate of 2.65 sec^{-1} would give a value of approximately 22% (Curtis, 1969). This virtually 10-fold difference in the adhesive abilities between the tissue types may be an indication of a difference in the type of adhesion which these two tissues possess.

Finally, the results show that cells aggregated in media of differing electrostatic dielectric constant do have their adhesive properties altered. In both cell types there can be clearly seen an increase in measured collision efficiency (adhesiveness) in media containing glycine and diglycine. To directly compare the effect in the two tissues, force constants were calculated for a series of aggregations performed in these media (Table 3). From these figures it can be shown that a difference covering nearly three

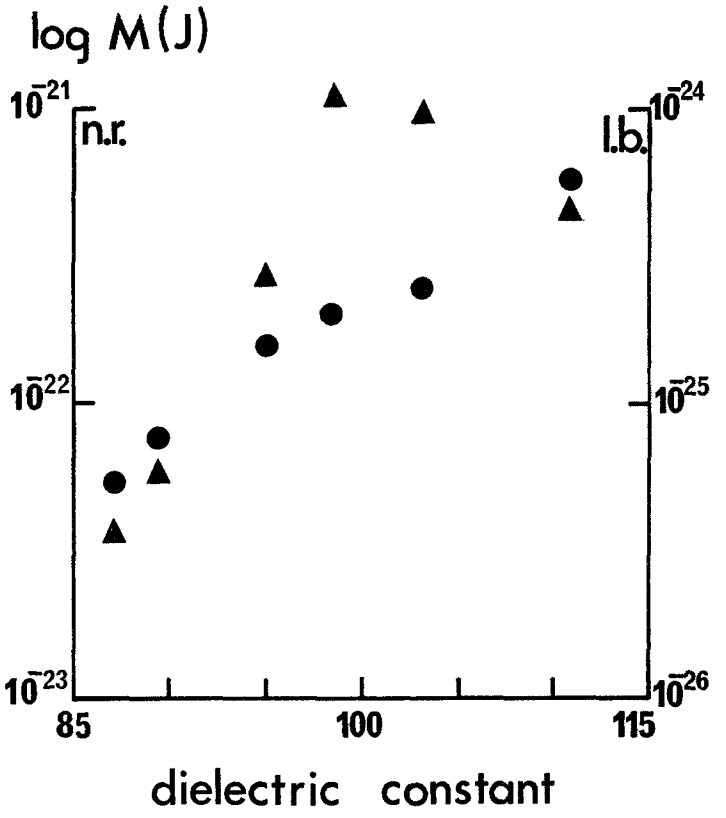

Fig. 1. Variation of force constant M with dielectric constant. • neural retina; ▲ limb bud. Left-hand abscissa (n.r.), neural retina; right-hand abscissa (l.b.), limb bud

orders of magnitude exists between the two cell types, neural retina having the higher values of M; a graphical representation is given in Fig. 1. It appears that there is a steady increase in the force constant with measured dielectric constant irrespective of which compound, glycine or diglycine, is used to modify this parameter, the dielectric constants given in Fig. 1 being a mixture of readings from both compounds. Neural retina cells seem to be less sensitive to changes in the dielectric constant than limb bud cells. If the figures recorded for aggregations in 1.0 M glycine are ignored (as these are suspect owing to observations of cell injury) then the interpretation of the results may be as follows. Both tissues show increases in force constant with dielectric constant, the rate of which seems to fall off when the latter rises above approximately 95.

This conclusion seems to contradict the proposed theoretical effects of dielectric constant on the role of electrostatic forces in cell adhesion (Brooks *et al.*, 1967). From the theory of particle interactions it can be shown that an increase in the electrostatic dielectric constant of the intervening medium

would lead to a gain in the potential energy barriers, resulting in an enhancement of the repulsive term of the total interaction energy between the particles (Weiss, 1967; Gingell, 1971). As a consequence there would be a decrease in the adhesive properties of the aggregating system rather than the increase seen in this study.

A possible explanation of the discrepancy between the predicted and experimental findings may be arrived at if one not only considers the effect of glycine and diglycine on the repulsive energy but also their effects on the attractive energy of adhesion. Clearly, if the energy of attraction were to increase by a greater extent than the energy of repulsion, the total interaction energy would also increase. This possibility may exist in the dielectric constant range investigated here, where the repulsive energy has not been significantly altered as judged by zeta potential measurements, but where additions of fairly large quantities of glycine and diglycine to the suspension media may have altered the attractive energy. It has previously been mentioned that the dielectric properties of the medium can affect the energy of attraction (Introduction) and the treatment of Dzyaloshinskii *et al.* (1960) for bodies at large separation distances can be used to exemplify this effect. The treatment utilizes the static dielectric constant only and if both bodies are identical the expression can be put

$$F=\frac{\hbar c}{L^4}\frac{\pi^2}{240\varepsilon_{30}^{\frac{1}{2}}}\left(\frac{\varepsilon_{10}-\varepsilon_{30}}{\varepsilon_{10}+\varepsilon_{30}}\right)^2\varphi\left(\frac{\varepsilon_{10}}{\varepsilon_{30}}\right) \tag{6}$$

where F denotes the force of attraction, $\hbar$ is Planck's constant/2π, L is the distance of separation, c is the velocity of light and ε_{10} and ε_{30} are the static dielectric constants of the particles and the intervening medium, respectively. The function $\varphi(x)$ has numerical values which are given in Fig. 4 of Lifshitz (1956). This function has a limiting value of 0.35 which was used in the following calculations. Taking an arbitrary value of $\varepsilon_{10}=50$ the use of Eq. (6) gives an F value of 3.56×10^{-27} J/L^4 when ε_{30} is 87. When ε_{30} is raised to 107 (covering the measured range of dielectric constant) then F is increased to 5.8×10^{-27} J/L^4. In fact the dielectric constant of the cell membrane is much lower than the figure of 50 used here (Curtis, 1967) so the effective increase in the force of attraction may be even greater. No calculations can be employed to show this, however, because when the difference between ε_{10} and ε_{30} is greater than a factor of 5 the function $\varphi(x)$ cannot be taken as the limiting value but is increased greatly (*see* Fig. 4 of Lifshitz, 1956). This will result in even greater values of F but at present the rise in the function is a little uncertain making further calculation pointless. The force of attraction as given by Eq. (6) is directly related to the

Hamaker constant (Gregory, 1969) so that from Eq. (2) it is seen that a rise in F values leads directly to an increase in attractive energy as visualized in the DLVO theory.

It has been mentioned that neural retina cells, which have a larger interaction energy M than limb bud cells, are not so greatly affected by changes in the dielectric constant of the media. An explanation for this, using the DLVO theory, may be that limb bud cells rest in a shallow secondary minimum, requiring only small variations in the properties of the medium to alter adhesive abilities by a large extent (as measured by the collision efficiency). The neural retina tissue, having much higher collision efficiencies may either lie in a deep and stable secondary minimum, a situation relatively insensitive to small changes in the Hamaker constant (Gingell, 1971), or possibly rest in the primary minimum.

The conclusions presented in this communication on cell membrane interactions are more complex than was originally supposed under the DLVO theory when energies of repulsion alone were considered. In previous studies, the effects of added components in the suspension media on the attractive force were either ignored (Pollack *et al.*, 1965) or taken into account by simply calculating potential energy curves over a series of differing Hamaker constants (Brooks *et al.*, 1967). The observed increases in the force constant M with dielectric constant may reflect a modification of the Hamaker constant which can be visualized with the aid of the Lifshitz theory.

The interpretation of the results is probably not as simple as the presented analysis suggests. The contribution of finite frequency components to the attractive force should also be discussed before concluding that the differences in kinetics of cell adhesion described in this paper can be convincingly attributed to modification of long range forces. It is hoped that this avenue of thought will later be extended to include a discussion of finite frequency terms.

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References

Abramson, H. A., Moyer, L. S., Gorin, M. H. 1942. Electrophoresis of Proteins. Reinhold Publishing Co., New York

Allgén, L.-G., Roswall, S. 1954. A dielectric study of a carboxymethylcellulose in aqueous solution. *J. Polymer. Sci.* **12**:229

Brooks, D. E. 1971. Theoretical and experimental studies on the interaction of neutral polymers with cell surfaces. *Biophys. Soc. Abstr.* **15** (TPM-G16):65a

Brooks, D. E., Millar, J. S., Seaman, G. V. F., Vassar, P. S. 1967. Some physiochemical factors relevant to cellular interactions. *J. Cell Physiol.* **69**:155

Brooks, D. E., Seaman, G. V. F. 1972. Electroviscous effect in dextran-erythrocyte suspensions. *Nature* **238**:251

Brooks, D. E., Seaman, G. V. F. 1973. The effect of neutral polymers on the electrokinetic potential of cells and other charged particles. 1. Models for the zeta potential increase. *J. Colloid Interface Sci.* **43**:670

Buckley, F., Maryott, A. A. 1958. Tables of dielectric dispersion data for pure liquids and dilute solutions. National Bureau of Standards Circular 589. U.S. Government Printing Office, Washington, D.C.

Collins, M. 1966. Electrokinetic properties of dissociated chick embryo cells. I. pH-surface charge relationship and the effect of calcium ions. *J. Exp. Zool.* **163**:23

Curtis, A. S. G. 1966. Cell adhesion. *Sci. Prog. Oxf.* **54**:61

Curtis, A. S. G. 1967. The Cell Surface. Logos & Academic Press, London

Curtis, A. S. G. 1969. The measurement of cell adhesiveness by an absolute method. *J. Embryol.* **22**:305

Curtis, A. S. G., Greaves, M. F. 1965. The inhibition of cell aggregation by a pure serum protein. *J. Embryol.* **13**:309

Curtis, A. S. G., Hocking, L. M. 1970. Collision efficiency of equal spherical particles in a shear flow. The influence of London-Van der Waals forces. *Trans. Faraday Soc.* **66**:1381

Derjaguin, B. V., Landau, L. D. 1941. Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. *Acta Physicochim., U.R.S.S.* **14**:633

Dzyaloshinskii, I. E., Lifshitz, E. M., Pitaevskii, L. P. 1960. Van der Waals forces in liquid films. *Soviet Phys. J.E.T.P.* **37**(10):161

Fricke, H., Curtis, H. J. 1937. The dielectric properties of water-dielectric interphases. *J. Phys. Chem.* **41**:729

Gingell, D. 1971. Computed force and energy of membrane interaction. *J. Theoret. Biol.* **30**:121

Gregory, J. 1969. The calculation of Hamaker constants. *Advanc. Colloid Interface Sci.* **2**:396

Handbook of Chemistry and Physics. 1966. 47th Edition. Chemical Rubber Publishing Co., Ohio

Haydon, D. A., Seaman, G. V. F. 1962. An estimation of the surface ionogenic groups of the human erythrocyte and of *Escherichia coli. Proc. Roy. Soc.* (*A*) **156**:539

Jones, G. E. 1972. Studies on Intercellular Adhesion. Ph. D. Thesis. University of Glasgow, Scotland

Kruyt, H. R., editor. 1952. Colloid Science, Vol. I. Elsevier Publishing Co., Amsterdam

Lifshitz, E. M. 1956. The theory of molecular attractive forces between solids. *Soviet Phys. J.E.T.P.* **29**(2):73

Mandel, M. 1965. Comments on impedance measurements of protein solutions. *Proc. Colloquium Protides of Biological Fluids.* **13**:415

Maryott, A. A., Smith, E. R. 1951. Table of dielectric constants of pure liquids. National Bureau of Standards Circular 514. U.S. Government Printing Office, Washington, D.C.

Moscona, A. A. 1961. Rotation-mediated histogenic aggregation of dissociated cells. *Exp. Cell Res.* **22**:455

Moscona, A. A. 1962. Analysis of cell recombinations in experimental synthesis of tissues *in vitro. J. Cell Comp. Physiol.* **60** (suppl. 1):65

Ninham, B. W., Parsegian, V. A. 1970. Van der Waals forces: Special characteristics in lipid-water systems and a general method of calculation based on the Lifshitz theory. *Biophys. J.* **10**:646

Oncley, J. L. 1938. Studies of the dielectric properties of protein solutions. I. Carboxyhaemoglobin. *J. Amer. Chem. Soc.* **60**:1115

Parsegian, V. A., Ninham, B. W. 1969. Application of the Lifshitz theory to calculation of Van der Waals forces across thin lipid films. *Nature* **224**:1197

Pethica, B. A. 1961. The physical chemistry of cell adhesion. *Exp. Cell Res., Suppl.* 8, p. 123

Pollack, W., Hager, H. J., Reckel, R., Toren, D. A., Singher, H. O. 1965. A study of the forces involved in the second stage of haemagglutination. *Transfusion* **5**:158

Schwan, H. P. 1963. Determination of biological impedances. *In:* Physical Techniques in Biological Research. W. L. Nastuk, editor. Vol. 6B, p. 323. Academic Press, London

Schwan, H. P., Maczuk, J. 1960. Simple technique to control the stray field of electrolytic cells. *Rev. Sci. Instrum.* **31**:59

Seaman, G. V. F. 1965. Electrophoresis using a cylindrical chamber. *In:* Cell Electrophoresis. E. J. Ambrose, editor. p. 4. J. and A. Churchill Ltd., London

Shaw, T. H. 1942. The elimination of errors due to electrode polarisation in measurements of the dielectric constants of electrolytes. *J. Chem. Phys.* **10**:609

Steinberg, M. S. 1964. The problems of adhesive selectivity in cellular interactions. *In:* Cellular Membranes in Development. M. Lock, editor. p. 321. Academic Press, London

Verwey, E. J. W., Overbeek, J. Th. G. 1948. Theory of the Stability of Lyophobic Colloids. Elsevier Publishing Co., Amsterdam

Weiss, L. 1967. The Cell Periphery, Metastasis and other Contact Phenomena. North-Holland Publishing Co., Amsterdam

Wilkins, D. J., Ottewill, R. H., Bangham, A. D. 1962*a*. On the flocculation of sheep leucocytes. I. Electrophoretic studies. *J. Theoret. Biol.* **2**:165

Wilkins, D. J., Ottewill, R. H., Bangham, A. D. 1962*b*. On the flocculation of sheep leucocytes. II. Stability studies. *J. Theoret. Biol.* **2**:176

Wyman, J., McMeekin, T. L. 1933. The dielectric constant of solutions of amino acids and peptides. *J. Amer. Chem. Soc.* **55**:908

Young, S. E., Grant, E. H. 1968. Measurements of permittivity of some biological solutions at frequencies below 1 MHz. *J. Sci. Instrum.* **1**:429