Molecular Aspects of Biological Surfaces

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1 Introduction

The boundary between two homogeneous phases, *e.g.* solid/electrolyte, commonly referred to as the surface, must be regarded as a thin film of characteristic thickness and not as a simple geometric plane on either side of which extend the homogeneous phases. The material in this surface phase shows properties which differ from those of the materials in the bulk phases; *e.g.* the excess or surface conductivity exhibited at a solid/electrolyte interface. The definition of a surface and its exact location becomes even more difficult for biological cells where the walls are freely permeable to water, ions, and small molecules. The surface here is envisaged as a series of shells which merge into one another; the outermost layer is an ionic atmosphere held in the neighbourhood by ionic groups on the cell surface. Inside this, for a bacterial cell, there may be a capsule within which lies the actual cell wall. Any process of separation or purification disrupts the fundamental parts, which are therefore degraded; this occurs whenever macromolecular material is separated from intact cells. The properties of interfaces must therefore be studied under such conditions that disorganization or degradation is minimal.

Particles of inorganic material, *e.g.* barium sulphate, examined by light or electron microscopy undergo no significant deformation. However, gross deformation occurs during the preparation and examination of biological cells by such techniques; interpretation of the results is therefore difficult. Surface structures of cells may be examined by the replica technique or by freeze-etching; using this latter technique, a distinctive pattern of rodlets has been observed on the surface of *Penicillium* conidia. Such techniques give no information about the nature, quantity, or arrangement of ionizable groups or surface lipid.

The technique of particulate electrophoresis, in which particles or cells are examined in free suspension, has provided valuable information on the properties of surfaces. The 'surface' studied by this technique is not that observed microscopically but is a region, within a few ångströms of the actual surface, defined in terms of those ions at the surface or those ionogenic components of the peripheral zone of the cells which contribute to the electrokinetic properties of the particle or cell under the experimental conditions of measurement. For biological cells, this zone is considered to be the outer portion of the cell membrane where the volume not occupied by macromolecular structures is accessible to ions and small molecules.

For non-ionogenic particles such studies have provided information on the structure of the double layer, colloid stability, the nature of the surface, particle–particle interaction, and the adsorption of macromolecules and polyions onto negatively charged surfaces. The role of the biological cell surface in immunology is now widely accepted, and the phenomena of invasion and metastasis may well depend on the chemical nature of the malignant cell membrane. Membrane surface components may be targets for the radiological damage of the cell. Changes in the surface properties of bacterial cells accompany changes in antibiotic resistance patterns and changes in environmental conditions. The interpretation of such observations depends on a detailed knowledge of the chemical composition and arrangement of components at the cell surface. It is for such reasons that extensive electrokinetic studies of biological cells in suspension have been made.

2 Theory

Electrokinesis is the general term applied to a group of phenomena which have a common origin in the asymmetrical distribution of ions at an interface; the electrical double layer. At an interface between two phases, on the time average, ions of one kind will be predominantly associated with one phase and those of the other kind with the other phase. The application of an electric field across such a system causes a tangential movement of one phase relative to the other at a velocity which depends on the potential at the plane of shear, the ζ-potential. This potential must be clearly distinguished from the thermodynamic or electrode potential and membrane potentials which exist when a porous membrane separates two electrolyte solutions.

Particles in suspension acquire a surface charge either by the adsorption of ions or by the ionization of surface charged groups. The system comprising electrolyte and particles in suspension is electrically neutral; however, each particle is much larger than a simple ion and so the net charge is many times greater than that of an ion.

The structure of the double layer, now widely accepted, is that developed by Stern, embodying the principles of Helmhlotz, Perrin, Gouy, and Chapman; this has been extensively reviewed.[1,2] The electrical double layer is divided into two parts: the Stern layer in which the charging ions and some gegenions held by specific ion adsorption remain almost in contact with the surface; in this layer there is a sharp fall in potential from ψ_0 at the surface to ψ_δ (Figure 1). The second part, which extends into the liquid phase, is diffuse; the potential falls from ψ_δ to zero. In this diffuse atmosphere, where thermal agitation permits free movement of ions, the distribution of positive and negative ions is not uniform since the electrostatic field at the surface results in a net attraction of ions of the opposite kind. The potential change in the Stern layer increases with concentration and valence type of the electrolyte; with multivalent counterions, charge-reversal can occur within the Stern layer (*i.e.* ψ_0 and ψ_δ have opposite signs).

[1] D. A. Haydon, *Recent Progr. Surface Sci.*, 1964, **1**, 94.
[2] J. T. Davies and E. K. Rideal, 'Interfacial Phenomena', Academic Press, New York, 1961.

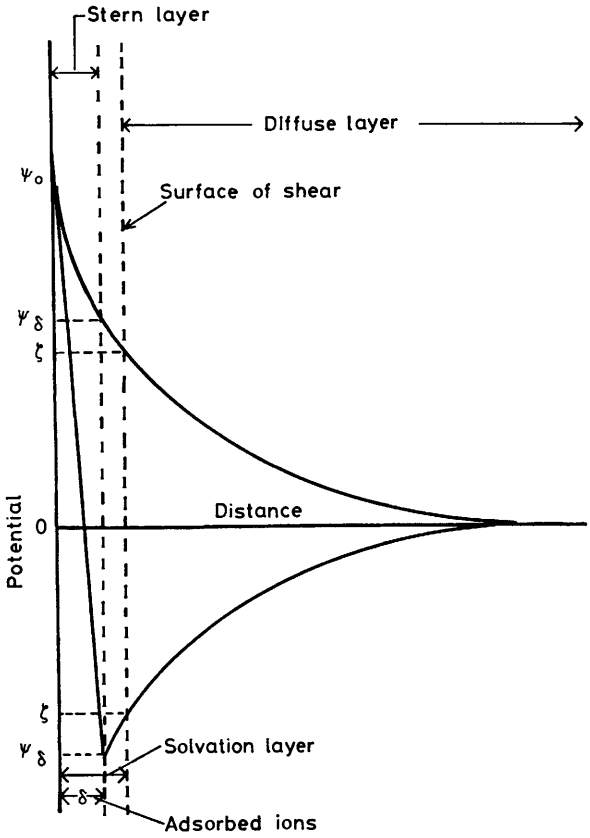

Figure 1 *Potential decay curve for an electrical double layer associated with a particle of surface potential ψ_0. The lower curve results from strong adsorption in the Stern layer*

When an electric field is applied to a particle in suspension, the migration velocity, v, is related to that part of the potential gradient across the shearing plane where the potential is ζ (*i.e.* the electrokinetic potential). The Smoluchowski equation:

$$\bar{v} = \frac{v}{X} = \frac{\epsilon_r \epsilon_0 \zeta}{\eta} \tag{1}$$

which relates the electrophoretic mobility, $\bar{v}$, to the ζ-potential (where ϵ_0 is the permittivity of a vacuum and ϵ_r and η are the relative permittivity and dynamic viscosity of the suspension medium, respectively), is valid for a cylindrical particle moving parallel to the field for all values of κa and for a spherical particle or a cylindrical particle moving at right angles to the field provided that $\kappa a > 100$. κ is the reciprocal thickness of the double layer, which at 298 K in water is:

$$\kappa/\mathrm{m}^{-1} = 1.034 \times 10^8 \, [I/(\mathrm{mol\ m}^{-3})]^{\frac{1}{2}} \tag{2}$$

and a is the radius of the particle and I the ionic strength of the electrolyte solution. For a particle of radius 1 μm, equation (1) may be applied provided that $I > 1$ mol m^{-3}; under other conditions, corrections for the distortion of the applied electric field have to be taken into account.[1,3] Equation (1) is valid for bacteria and cells in suspension in electrolyte solutions at $I > 1$ mol m^{-3}, provided that a is taken as the radius of the particle and not the radius of the fine structure on the cell surface. Corrections for surface conductance are negligible for particles the size of biological cells.

The surface charge density, σ, for a rigid surface impenetrable to counterions in a symmetrical electrolyte with ions of charge z and concentration c is given by:

$$\sigma = \left[\frac{N_A \epsilon_0 \epsilon_r kTc}{125}\right]^{\frac{1}{2}} \sinh (ze\zeta/2kT) \qquad (3)$$

in which ζ (the potential at the shear plane) replaces ψ_δ (the potential at the outside of the Stern layer), and where N_A is the Avogadro constant, k the Boltzmann constant, e the electronic charge, and T the temperature. For intact cells this is not a realistic model since the outermost surface is porous to ions. For such systems the value given by equation (3) underestimates the charge density by a factor of $[1 + (1 - \alpha)^{\frac{1}{2}}]$, where α is the fraction of the total space within the surface that is not available to counterions.[4] When the surface is completely penetrable to counterions, $\alpha = 0$, and the charge density is twice that given by equation (3). In the absence of proper values of α it is not possible to use this correction in calculations of the surface charge density.

Using the accepted values of the physical constants, the ζ-potential is calculated from the measured electrophoretic mobility ($x \times 10^{-8}$ m^2 s^{-1} V^{-1}) using equation (1), which becomes:

$$\zeta/\text{mV} = 12.85x \qquad (4)$$

and the charge density [equation (3)] from the ζ-potential:

$$\sigma/\text{C m}^{-2} = 3.713 \times 10^{-3}(c/\text{mol m}^{-3})^{\frac{1}{2}} \sinh [(\zeta/\text{mV})/51.3] \qquad (5)$$

or

$$= [1 + (1 - \alpha)^{\frac{1}{2}}]\, 3.713 \times 10^{-3}\, c^{\frac{1}{2}} \sinh [\zeta/51.3] \qquad (5a)$$

The use of equations (4) and (5) is complicated by the relationship between ζ and ψ_0 or ψ_δ and by the fact that η and ϵ_r of the medium may not be the same as for the bulk but may have abnormal values within the double layer. For surfaces with $\sigma < 1$ C m^{-2}, a good approximation may be obtained by assuming the bulk value of the ϵ_r/η ratio.[1]

Associated with the high concentration of ions near a charged surface will be an additional conductance, the surface conductance; this may reduce the value of the electrophoretic mobility by a partial short-circuiting mechanism. Since the

[3] C. J. van Oss, *Sepn. and Purification Methods*, 1975, **4**, 167.
[4] D. A. Haydon, *Biochim. Biophys. Acta*, 1961, **50**, 450.

measured surface conductance is 100 to 1000 times greater than the calculated contribution from the diffuse part of the double layer, the greater proportion of the surface conductance can be assigned to the fixed part of the double layer.[5] It is not usual to make allowance for surface conductance in the calculation of ζ-potentials for large particles in suspension (where the error involved is less than the experimental error in the measurements).

On account of these uncertainties, most workers prefer to record and report electrophoretic mobility values and to discuss their results in terms of these experimentally measured quantities. Under standard conditions of measurement (*i.e.* κa constant) a comparison of mobility values (same sign as ζ) of different particles in suspension is equivalent to comparing the ζ-potentials.

3 Experimental Techniques

In the particulate micro-electrophoretic technique, the rate of migration of an individual particle (viewed under a microscope) is measured under a known applied electric field at a fixed temperature. The technique is rapid and can be used with suspension media (usually aqueous) over a wide range of pH and ionic strength. High-power magnification (up to 600 times) is used and thus the shape, size, and orientation of the particle and the presence of mixed populations (with regard to nature, size, or surface properties) can be observed in an environment which is constant with respect to pH and ionic strength over the small time (< 5 s) required for a measurement.

Basically the apparatus consists of a closed transparent chamber (cylindrical or rectangular), mounted between two reversible non-gassing electrodes, in which a suspension can be observed microscopically under controlled conditions. The development of the apparatus has been extensively reviewed.[6–8] Many of the earlier observation chambers do not conform to the necessary requirements; results obtained in such apparatus should be interpreted with caution. Full details of the experimental procedures and precautions necessary to obtain reproducible results have been described for rectangular chambers[8] and for cylindrical chambers.[7,8] Of the commercially available instruments the Zeiss Cytopherometer and the Rank Particulate Electrophoresis Apparatus Mark II most nearly conform to the requirements.[9]

The main errors in the measurement of the electrophoretic mobility of particles in suspension arise from the inaccurate location of the stationary level within the chamber, incorrect focusing of particles at the stationary level, the presence of sedimented particles (particularly serious with cylindrical cells and rectangular cells mounted horizontally), polarization of the electrodes, timing, Brownian movement of the particles, and variation within the sample. For a large number of different systems, including suspensions of biological cells, the

[5] M. H. Wright, G. E. Nichols, and A. M. James, *Kolloid Z. Z. Polymere*, 1971, **245**, 525.
[6] A. M. James, *Progr. Biophys. and Biophys. Chem.*, 1957, **8**, 98.
[7] G. V. F. Seaman, in 'Cell Electrophoresis', ed. E. J. Ambrose, J. and A. Churchill. London, 1965, p. 48.
[8] A. M. James, *Surface and Colloid Sci.*, 1979, **11**, 121.
[9] L. S. Moyer *J. Bacteriol.*, 1936, **31**, 531.

confidence limit of a single mean is $\pm 3\%$ at $p = 0.05$; this means that two surfaces with mobility values (determined under identical conditions) differing by more than 10% have significantly different surface properties.

A technique has been described[10] in which the ζ-potential of a film of cells coated on the inside of a glass capillary tube can be calculated from a measurement of the electro-osmotic velocity of a buffer solution through the capillary. It is claimed that the value so obtained is for cells which have not undergone changes in ζ-potential, viability, or morphology, which could result from the standard methods of pretreatment used to prepare suspensions for microelectrophoresis. The value obtained, however, is an average value for a film of cells held in a particular orientation at the glass surface and is not for individual cells in free suspension. Using this technique, it is not possible to detect electrokinetically heterogeneous cell populations.

The most difficult problem in the study of surface properties is the establishment of a clean and reproducible surface; contamination may be due to adsorbed ions on non-ionogenic surfaces, adsorbed ions or macromolecules (such as waste products and toxins from the growth medium) on bacterial and yeast cells, or adsorbed plasma and serum proteins on animal cells. The washing procedure must be such that nothing is added to nor removed from the surface[8] and the final surface must be reproducible. Only when such criteria have been established can experimental results be interpreted with any degree of confidence. The problem of purity of materials and preparation of the suspension has been discussed for octadecanol suspensions,[11] for dispersions of model compounds of biological interest,[12] for silver iodide sols,[13] for suspensions of monodisperse polystyrene latices,[14] and for kaolinite particles.[15] The care required in the preparation of suspensions of biological cells is generally considered for each system under study.

4 Surface Properties of Non-ionogenic Particles

To ensure the stability of hydrophobic sols against aggregation, the particles must carry an electric charge, which must be distributed correctly; good stability requires extended double layers. Much of the data obtained from electrokinetic studies of non-ionogenic systems has been used in the understanding of colloid stability.[16] Ideally, to provide a complete test of the theories, measurements of the Stern potential, ψ_δ, are required. This is not a practical possibility and instead the value of the ζ-potential is determined; this quantity provides a useful measure of the part of the potential drop within the double layer, and, moreover, as the potential drop becomes small, the reasonable assumption can be made that ζ and ψ_δ are the same. The electrophoretic mobility of particles with non-ionogenic

[10] R. M. Fike and C. J. van Oss. *In Vitro*, 1976, **12**, 428.
[11] S. Hollingshead. G. A. Johnson. and B. A. Pethica, *Trans. Faraday Soc.*, 1965, **61**, 577.
[12] J. N. Mehrishi and G. V. F. Seaman, *Trans. Faraday Soc.*, 1968, **64**, 3152.
[13] R. H. Ottewill and A. Watanabe, *Kolloid Z.*, 1960, **170**, 38.
[14] R. H. Ottewill and J. N. Shaw. *Kolloid Z. Z. Polymere*, 1967, **215**, 161.
[15] D. J. A. Williams and K. P. Williams, *J. Colloid Interface Sci.*, 1978, **65**, 79.
[16] J. Th. G. Overbeek, *J. Colloid Interface Sci.*, 1977, **58**, 408.

surfaces is dependent on the nature of the counterions and on their concentration; attempts have been made to explain the results in terms of the effect of the ions on the properties and structure of the water in the double layer.

The stability of negatively charged silver iodide sols in the presence of cationic surface-active agents[17] and of positively charged silver iodide sols in the presence of anionic surface-active agents[18] is correlated with the ζ-potential of the particles. For each type of system the stability of the sol is minimal when the ζ-potential is low and increases as the ζ-potential becomes progressively more positive or negative. Combination of the data from electrokinetic and stability studies gives curves of log W (W is the stability ratio) against ζ (Figure 2) which are in good agreement with theoretical predictions, indicating that coagulation is determined by a potential-energy barrier; the calculated Hamaker constants are in the range 10^{-19}—10^{-20} J. Similar results have been reported for the stability of monodisperse polystyrene latex dispersions,[19] of rubber latex sols,[20] and of graphitized carbon blacks.[21]

The decrease of stability of silica sols with increasing pH, *i.e.* increasing surface charge,[22] seems at variance with the DLVO theory; since, at low pH values, the absolute coagulation concentrations exceed the values normally found for hydrophobic sols by orders of magnitude, it is apparent that hydration must play an important role in stabilizing such systems. A modification of the DLVO theory[23] which takes into account the influence of adsorbed layers of different composition on the stability indicates that just one layer of water would be sufficient to account for the observed trend of stability with pH. At the present time, insufficient data are available to test this hypothesis fully.

A study of the surface properties of the barium sulphate suspensions used as *X*-ray-opaque media and their interaction with the mucosal lining of the stomach is an interesting application of the electrokinetic technique. The electrical properties of commercial preparations of such barium sulphate suspensions are quite unlike those of normal barium sulphate suspensions[24] (Figure 3). The barium sulphate particles are coated with hydrocolloids which are largely carboxyl in nature; these coated particles exhibit the properties of a typical carboxyl surface (compare Figures 3 and 6). The hydrocolloid in these suspensions is present as a stabilizing agent; there is no correlation between the charge carried by the particles and the stability of the preparation. Since the particles are uncharged at low pH values, the possibility that attachment to the mucosal lining is electrostatic in nature is most remote. In the ileum of a rat the particles become coated with mucosa and adhere to the mucosal membrane, as revealed by the different pH–mobility curves. When denaturation of the mucosal material occurs

[17] R. H. Ottewill and M. C. Rastogi, *Trans. Faraday Soc.*, 1960, **56**, 880.
[18] R. H. Ottewill and A. Watanabe, *Kolloid Z.*, 1960, **170**, 132.
[19] R. H. Ottewill and J. N. Shaw, *Trans. Faraday Soc.*, 1966, **42**, 154.
[20] C. G. Force and E. Matijević, *Kolloid Z. Z. Polymere*, 1968, **224**, 51.
[21] G. D. Parfitt and N. H. Picton, *Trans. Faraday Soc.*, 1968, **64**, 1955.
[22] J. Depasse and A. Watillon, *J. Colloid Interface Sci.*, 1970, **33**, 430.
[23] J. Lyklema, *Croat. Chem. Acta*, 1977, **50**, 77.
[24] R. J. Simmonds and A. M. James, *Cytobios*, 1976, **15**, 191; 1976, **16**, 107.

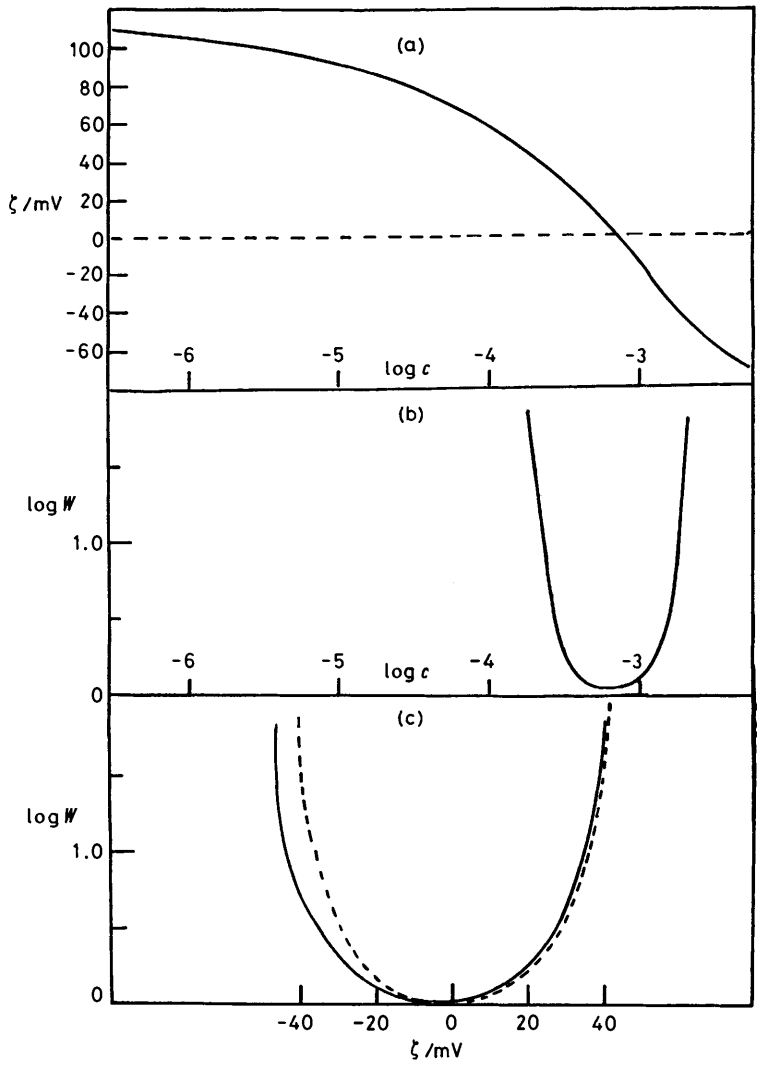

Figure 2 *Electrophoretic behaviour and stability of positive silver iodide sols in the presence of sodium decyl sulphate*;[13,18] (a) ζ *against* log *c*; (b) log *W against* log *c* (*W is the stability ratio*); (c) log *W against* ζ, (- - - - - -) *theoretical curve*, (———) *experimental curve*

there is decreased adsorption onto the membrane; this leads to poor coverage and hence the possibility of poor quality *X*-ray pictures.[24]

The negative charge on particles prepared from human enamel is due to the presence of surface sialic acid moieties. Treatment of these particles with chlorhexidine, an inhibitor of plaque formation, results in a reduction of the negative charge, indicating the adsorption of chlorhexidine on the surface. At this concen-

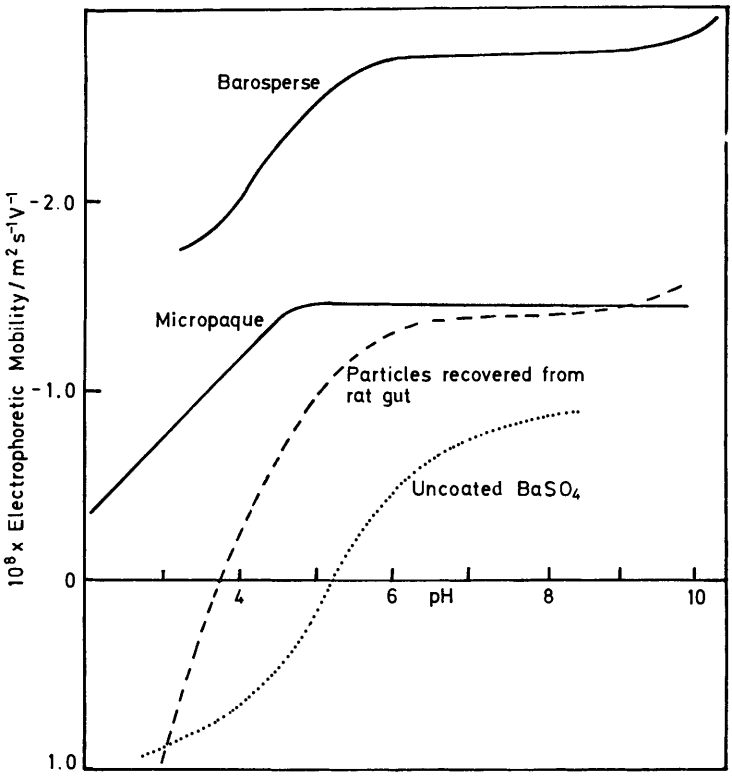

Figure 3 *pH–Mobility curves for various barium sulphate preparations (Micropaque and Barosperse are commercial barium meals used as X-ray opaques)*[24]

tration (2%), chlorhexidine inhibits the adhesion of Ehrlich ascites tumour cells *in vitro*. Further work is necessary before these studies *in vitro* can be extrapolated to the situation *in vivo*.[25]

5 Surface Properties of Ionogenic Particles

Biological cells constitute the most extensively studied and most important class of particles with ionogenic surfaces. The charge on such surfaces is due to the dissociation of ionogenic surface groups (*e.g.* amino, carboxyl, phosphate); there is no evidence of anion adsorption onto such surfaces.

The chemical methods of cell wall degradation (*e.g.* by acid or alkaline hydrolysis, solvent extraction) and the analysis of the fractions so obtained give a general idea of the nature and amounts of the different components present in the cell wall. This information does not lead directly to the arrangement of the

[25] M. E. Neiders and L. Weiss, *Arch. Oral Biol.*, 1972, **17**, 949.

macromolecules in the cell wall nor to the nature and arrangement of the surface components.

Many experimental difficulties arise in the preparation of cell suspensions, and it is essential that adequate precautions are taken and experimental conditions established for each type of cell so that the surface under study is undamaged and is reproducible. Cells must be washed free of growth medium, plasma, *etc.* which may contain salts, macromolecules, proteins, toxins, *etc.* that could be adsorbed; the washing must, however, not be so excessive that actual surface material is removed. The structural integrity of the cells must be preserved and lysis must not be allowed to occur since the products so released may be readsorbed onto the lysed cells. For blood and tissue cells it is often necessary to use solutions of high electrolyte or sucrose content to maintain an adequate osmotic pressure or to stabilize the surface chemically (treatment with aldehydes).

Although there is a large difference in size and a difference in the charge-determining groups between different biological cells (Table 1), nevertheless the

Table 1 *The electrophoretic mobility of different particles measured at* pH 7

Species	*Biological characters*	$10^8 \times$ *mobility* /$m^2\ s^{-1}\ V^{-1}$
Klebsiella aerogenes	fim+	−1.74
	fim−	−3.50
Staphylococcus aureus	methicillin-sensitive	−1.00
	methicillin-resistant	−1.48
	trained to methicillin	−1.50
Streptococcus pyogenes	Type 2G	−1.03
	Type 2M	−0.89
Human blood cells	Erythrocytes	−1.08
	Lymphocytes	−1.09
	Platelets	−0.85
Hamster kidney cells	Erythrocytes	−1.35
	Tissue cells	−0.65
	Tumour cells	−1.2
Erythrocytes	Chimpanzee	−1.18
	Chicken	−0.82
	Dog	−1.28
	Horse	−1.16
	Ox	−0.96
	Pig	−0.88
Chlorella cells		−1.70
Hydrogen ion		+36.70
Chloride ion		−6.8
Sodium ion		+5.2
Colloidal gold		−3.2
Oil droplets		−3.1

mobility values of the cells are all of the same order of magnitude; thus one single mobility value will not serve to characterize a particular cell or a particular surface group.

A. Bacteria.—There is no evidence for the polarity of individual organisms, with the exception of cells of *Spirochaeta*.[26] Under controlled conditions of growth and measurement, cells of a given strain have a reproducible mobility value; the value is not constant for all cells constituting a population but in general shows a Gaussian distribution about a mean. During repeated growth of cells in the presence of dyes,[6] mixed populations, in which cells have a range of mobility values, are observed; this indicates that the cells do not all respond to the presence of the dye to the same extent. The change from the fimbriate to the non-fimbriate state and *vice versa* of non-capsulate organisms is accompanied by a change in the surface charge. When fim+ cells of *Escherichia coli* grown in broth are repeatedly grown on solid medium, a bimodal population is first formed;[27] some cells have low mobility values characteristic of fim+, while others have higher mobility values, *i.e.* fim− cells. Repeated growth on agar results in the population becoming electrokinetically homogeneous, having the high mobility value (Figure 4).

The surface charge of some organisms, measured in suspension at constant pH and ionic strength, varies with the age of the cells. Although a change of mobility during the growth cycle can be attributed to changes in the nature of the surface, it is not necessarily correct to infer the opposite when there is no change of mobility. Cells of *E. coli* have a lower negative charge during the period of 'physiological youth' than at any other age.[28] In contrast, cells of *Klebsiella aerogenes* exhibit a slightly higher negative charge during the logarithmic growth phase; the change in the surface properties, attributed to changes in the amount of capsular polysaccharides and the size of the organisms, is more pronounced when the cells are pretreated with phenol. The increase in the negative charge of cells of *Strep. pyogenes* grown in liquid medium is due to the accumulation of surface hyaluronic acid (a 1:1 polymer of *N*-acetylglucosamine and glucuronic acid) during the active growth phase, followed by its removal during the stationary phase.[29] The charge is constant at all ages when hyaluronidase is present in the growth medium; hyaluronic acid is not detectable on the surface of these organisms. The increase in the negative mobility of cells of *Strep. pyogenes* during growth in the presence of glycerol is due to an increase in the amount of surface phosphatide lipid during the logarithmic growth phase; this is followed by a decrease during the stationary phase to a constant value.[30] The changes in the surface composition of vegetative cells of *Bacillus megaterium* during growth are reflected in changes in the electrophoretic mobility and sensitivity of the cells to

[26] M. T. Dyar and E. J. Ordal, *J. Bacteriol.*, 1946, **51**, 149.
[27] A. M. James and C. F. List, *Biochim. Biophys. Acta*, 1966, **112**, 307.
[28] L. S. Moyer, *J. Bacteriol.*, 1936, **32**, 433.
[29] M. J. Hill, A. M. James, and W. R. Maxted, *Biochim. Biophys. Acta*, 1963, **66**, 264.
[30] M. J. Hill, A. M. James, and W. R. Maxted, *Biochim. Biophys. Acta*, 1963, **75**, 414.

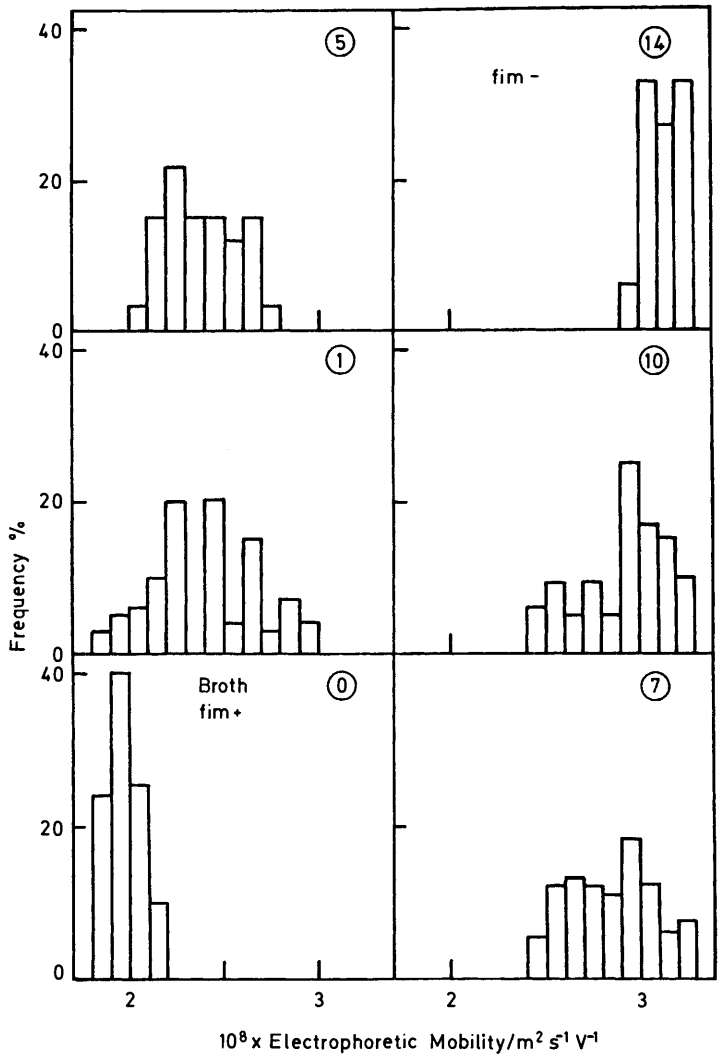

Figure 4 *The change in the electrophoretic mobility distribution of broth-trained cells of* E. coli *during serial subculture on agar (numbers refer to the number of subcultures on agar)*[27]

lysozyme.[31] Growth at different temperatures generally has no effect on the mobility of cells measured under standard conditions; the one exception is that of methicillin-resistant cells of *Staphylococcus aureus*.[32]

For cells of a fixed age, changes in the nature of the suspension medium (*e.g.*

[31] H. W. Douglas and F. Parker, *Biochem. J.*, 1958, **68**, 99.
[32] A. W. Hill and A. M. James, *Microbios*, 1972, **6**, 169.

ionic strength, pH, and the presence of surfactants) give rise to changes in the surface charge of the cells which can be used to give information about the nature and quantity of surface components.

Effect of Electrolyte Concentration of Suspension Medium. In electrolyte solutions of fixed pH, as the concentration increases the ζ-potential first becomes more negative, passes through a maximum value, and then decreases (Figure 5). As the ionic strength of the electrolyte solution is decreased, the thickness of the ionic atmosphere increases [equation (2)] and the Coulombic screening of the charged groups is reduced. In addition, ionic groups further from the surface of shear no longer possess counterions that move with the cell as a unit and are therefore unmasked; these then contribute to the total surface charge.

The concentration of electrolyte required for the particle to attain a zero charge is greatest for a univalent cation (usually this is an extrapolated value) and decreases with increasing valence of the cation. Thus the charge carried by a negatively charged particle may be completely reversed in the presence of a very low concentration of a thorium salt; this is the charge-reversal concentration. A 'reversal-of-charge spectrum', plotted from the measured variation of the ζ-potential of cells in various electrolyte solutions, can be compared with the corresponding spectra for naturally occurring substances. Using this method, several yeasts were classified as typical carboxyl colloids, the surface of *E. coli* was classified as an acidic polysaccharide, possibly an arabinate,[33] the surface of *Mycobacterium phlei* was shown to be partially phosphatide in nature,[34] and the principal surface groups of spores of *Bacillus megaterium*, *B. cereus*, and *B. subtilis* were characterized as carboxyl.[35]

The experimental data for the plotting of most charge-reversal spectra have been collected under conditions of varying ionic strength; with decreasing ionic strength, additional charged groups lying deeper in the cell wall will contribute to the total charge. This difficulty is overcome if the variation of mobility with the concentration of a range of electrolytes is studied in an electrolyte solution of constant ionic strength, *e.g.* by the addition of an indifferent electrolyte (KNO_3). Thus a more meaningful interpretation of the effect of the different cations on the total charge can be made. Apart from the very large number of measurements required over a wide range of concentrations, it is often necessary to extrapolate the ζ-potential–concentration curve to concentrations in excess of the highest concentration used in order to obtain the charge-reversal concentration; this gives rise to considerable errors. Some of the electrolytes, particularly those containing multivalent ions, undergo hydrolysis; this results in a change of pH and also a possible change in the character of the ionic species present in solution.

Cells of rough variants of some bacteria in solutions of high ionic strength have a higher ζ-potential than do cells of the smooth variant under the same conditions,[28] a difference which has been used to study the dissociation of bacterial

[33] J. T. Davies, D. A Haydon, and E. K. Rideal, *Proc. Roy. Soc.*, 1956, **B145**, 375.
[34] D. M. Adams and E. K. Rideal, *Trans. Faraday Soc.*, 1959, **55**, 185.
[35] H. W. Douglas, *J. Appl. Bacteriol.*, 1957, **20**, 390.

strains.[6] In solutions of low ionic strength the ζ-potentials of the two forms become the same (Figure 5).

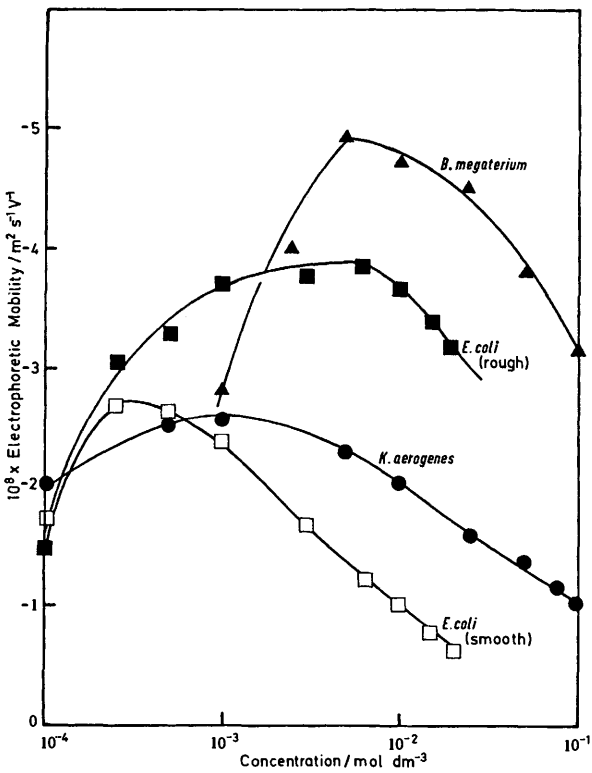

Figure 5 *ζ-Potential–concentration curves for various bacterial species.* (▲) Bacillus megaterium *in acetate–barbiturate buffer solution* (H. W. Douglas, *Trans. Faraday Soc.*, 1955, **51**, 146); (■) E. coli (*rough variant*) *in acetate buffer solution*;[28] (□) E. coli (*smooth variant*) *in acetate buffer solution*;[28] (●) Klebsiella aerogenes *in acetate–barbiturate buffer solution* (G. J. Gittens, Ph.D. Thesis, London, 1962)

Effect of pH of Suspension Medium. The pH of the surface of a particle is different from that in the bulk of the solution, particularly at low salt concentrations. The equation of Hartley and Roe:[6]

$$\text{pH(surface)} = \text{pH(bulk)} + \frac{e\,\zeta}{2.303\,kT} = \text{pH(bulk)} + A\,\bar{v} \qquad (6)$$

gives the pH at the plane of shear [A is a constant (0.217 at 25 °C), for particles of bacterial size].

The change of mobility of an ionogenic surface with the pH of the suspension medium (at constant I) is essentially a titration of the charged surface groups. Care must be exercised to ensure that the biological surface is not damaged or

denatured by suspension in buffer solution of adverse pH. Typical pH–mobility curves for model surfaces (Figure 6) are of use in the interpretation of the pH–mobility curves for biological cells.

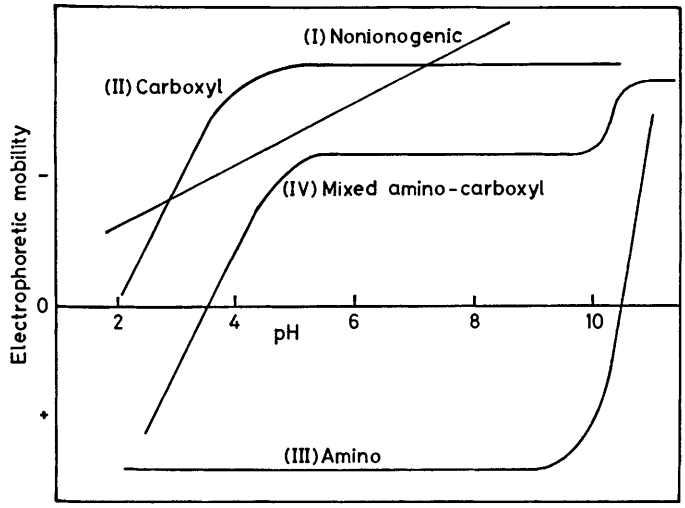

Figure 6 *Typical pH–mobility curves obtained for various model and biological surfaces*

Curve I is typical of a non-ionogenic surface and represents the adsorption of anions and/or the desorption of protons with increase of pH; such behaviour has been reported for polystyrene latex particles, Nujol oil droplets,[36] and suspensions of octadecanol. Deviations from linearity are generally attributed to the presence of traces of surface impurities.

Curve II is typical of an ionogenic surface in which the charged group is anionic (carboxyl, sulphate, phosphate); experimental evidence suggests that on such surfaces the charge due to ion adsorption is negligible. Curves of this type for cells of *K. aerogenes*[37] are due to the presence of carboxyl groups; the mobility is constant at pH values in excess of pH 5, indicating complete ionization of the carboxyl groups. The pK of the surface, 2.8, is consistent with the presence of glucuronic acid, a known component of the capsule of this organism. Cells of *E. coli*,[33] with a pK of 2.9, and cells of slow-growing strains of *Rhizobium*,[38] with a pK of 2.3—2.5, have an exclusively carboxyl surface. The charge carried by cells of five variant strains of *Mycobacterium bovis* BCG, *M. smegmatis*, *M. microti*, and *M. phlei*, which have coincident pH–mobility curves and surface pK values in the rage 3.2—3.5, has been attributed to the phospho-diester groups which link the mycolic esters to the mucopeptide backbone of cells of these species of organism.[39]

[36] H. W. Douglas and D. J. Shaw, *Trans. Faraday Soc.*, 1958, **54**, 1748.
[37] G. J. Gittens and A. M. James, *Biochim. Biophys. Acta*, 1963, **66**, 237.
[38] K. C. Marshall, *Austral. J. Biol. Sci.*, 1967, **20**, 429.
[39] L. E. Hardham, Ph.D. thesis, London, 1979.

When the carboxyl groups of *K. aerogenes* were esterified with diazomethane or methanolic HCl,[37] cells with zero charge at pH values up to 7 were obtained. Cells of *E. coli* treated with a mixture of carbodi-imide and glycinamide[40] became net positively charged at pH values up to 8 due to the discharge of the carboxyl groups which dominated the surface.

Curve III is characteristic of an amino-type surface, which is positively charged at low pH values due to the presence of $—NH_3^+$; at higher pH values the proton is lost and the surface eventually becomes negatively charged, due presumably to anionic adsorption onto the polar nitrogen atom.

The mixed amino–carboxyl surfaces of a large number of bacteria give rise to pH–mobility curves of type IV, with a positive charge at low pH values due to $—NH_3^+$, a negative charge at intermediate pH due to the presence of both $—COO^-$ and $—NH_3^+$, and generally a higher, negative value at high pH values due to $—COO^-$ alone. The pH–mobility curves of *B. cereus*, *B. megaterium*, and *B. subtilis*, when compared with those obtained for different natural and synthetic polymers, indicate that the surfaces of the resting spores are composed essentially of differently oriented layers of the same hexosamine peptide.[36] The electrokinetic behaviour of cells, protoplast membranes, and defatted protoplast membranes of *Micrococcus lysodeikticus* is controlled by the ionization of carboxyl and amino groups, while the membrane lipid is characteristic of a phosphate-type colloid.[41] Although the bacterial form, the L-form envelope, and the protoplast of cells of *Strep. pyogenes* all exhibit properties typical of a mixed carboxyl–amino surface, nevertheless the relative contributions of the two groups are significantly different among the various species.[42]

Treatment of a mixed carboxyl–amino surface with toluene-*p*-sulphonyl chloride[43] or 1-fluoro-2,4-dinitrobenzene[44] blocks the amino groups and prevents their ionization; the pH–mobility curve of the resulting surface is characteristic of a simple carboxyl surface. This leads to an estimated value of the relative contributions of the carboxyl and amino groups to the total charge. For a range of strains of *Strep. pyogenes* the ratio of carboxyl/amino of the glossy variant is always greater than that of the matt variant. As the amino-acid composition of the surface antigens is the same, the essential difference between the surface protein antigens of the two variants must be in the extent of cross-linking in the protein.[44]

A study of the shapes and positions of the pH–mobility curves of FDNB-treated cells of *Strep. pyogenes* after treatment with specific decarboxylases led to the identification of the surface carboxyl groups as those of alanine and of α- and γ-glutamic acid, in the ratio 1:1:3.[45]

Marshall,[38] classifying root-nodule bacteria as either slow- or fast-growing, showed that the surface charged groups of the former were exclusively carboxyl,

[40] R. A. Neihof and W. H. Echols, *Physiol. Chem. and Phys.*, 1978, **10**, 329.
[41] A. V. Few, A. R. Gilby, and G. V. F. Seaman, *Biochim. Biophys. Acta*, 1960, **38**, 130.
[42] A. M. James, M. J. Hill, and W. R. Maxted, *Antonie van Leeuwenhoek J. Microbiol. and Serol.*, 1965, **31**, 423.
[43] H. W. Douglas, *Trans. Faraday Soc.*, 1959, **55**, 850.
[44] M. J. Hill, A. M. James, and W. R. Maxted, *Biochim. Biophys. Acta*, 1963, **75**, 402.
[45] M. J. Hill, A. M. James, and W. R. Maxted, *Biochim. Biophys. Acta*, 1963, **71**, 740.

whereas those of the fast-growing strains were amino and carboxyl. There is no relationship between nitrogen-fixing ability and electrophoretic mobility in strains of *Rhizobium meleloti*. In the presence of increasing concentrations of clays (sodium montmorillonite or illite) the mobility of cells of all strains approached the value characteristic of the clay particles,[46] indicating adsorption of clay particles onto the cells. The adsorption isotherms so obtained revealed that *Rhizobia* with a simple carboxyl surface adsorbed about twice as much clay as did the cells with the complex carboxyl–amino surface.

The shape and position of pH–mobility curves for cells of *Staph. aureus* grown at 37 °C (Figure 7a), not explainable in terms of the dissociation of ionogenic groups, depend on the sensitivity or resistance of the cells to methicillin[32] and, for methicillin-resistant cells, the shape and position of the curve depends on the temperature of growth[47] (Figure 7b). It is a well established fact that cultures of naturally occurring methicillin-resistant *Staph. aureus* grown at 43 °C are sensitive to methicillin and when grown at 25 °C are resistant to methicillin; at 37 °C the culture consists of a mixed population in which the majority of the cells are of normal sensitivity to methicillin, with about 1 in 10^5 having high resistance to methicillin. Despite this, all the cells of such a culture grown at 37 °C are electrokinetically homogenous, with a surface which is quite unlike that of cells of a sensitive strain. It is thus apparent that, in becoming resistant to methicillin, the surface properties of all the cells, irrespective of whether they are sensitive or resistant to methicillin, have undergone a marked change. The pH–mobility curves of methicillin-sensitive cells treated with sodium metaperiodate (Figure 7a), which degrades teichoic acid (a known wall component), are characteristic of a simple carboxyl surface; thus teichoic acid and carboxyl groups are among the surface components contributing to the charge of sensitive cells in suspension at pH 4—5. The phosphate/carboxyl ratio, calculated from the charge densities at pH 3.5 of the treated and untreated sensitive cells, is 2.2; for methicillin-resistant cells grown at 25, 37, and 43 °C the phosphate/carboxyl ratios are 0.4, 0.75, and 1.0 respectively. There is thus less teichoic acid on resistant cells than on sensitive cells; and further, the amount of surface teichoic acid is less on resistant cells grown at the lower temperature. In suspension at pH >7 the teichoic acid does not contribute to the surface charge, but as the pH decreases the teichoic acid undergoes conformational changes whereby the ionized phosphate groups are oriented into the liquid and so contribute to the surface charge, which then becomes more negative. At pH <3.5 the phosphate groups become protonated and the negative charge decreases. These changes in the conformation of the teichoic acid are purely physical in nature and are completely reversible over the pH range 3 to 8. The smaller amount of teichoic acid associated with methicillin-resistant cells, as compared to sensitive cells, gives rise to the abnormal pH–mobility curves which pass through a minimum value at pH 4.5.

Repeated growth of methicillin-resistant cells at 43 °C in the absence of antibiotic eventually produces cells which are sensitive to methicillin and which

[46] K. C. Marshall, *J. Gen. Microbiol.*, 1969, **56**, 301.
[47] A. W. Hill and A. M. James, *Microbios*, 1972, **6**, 157.

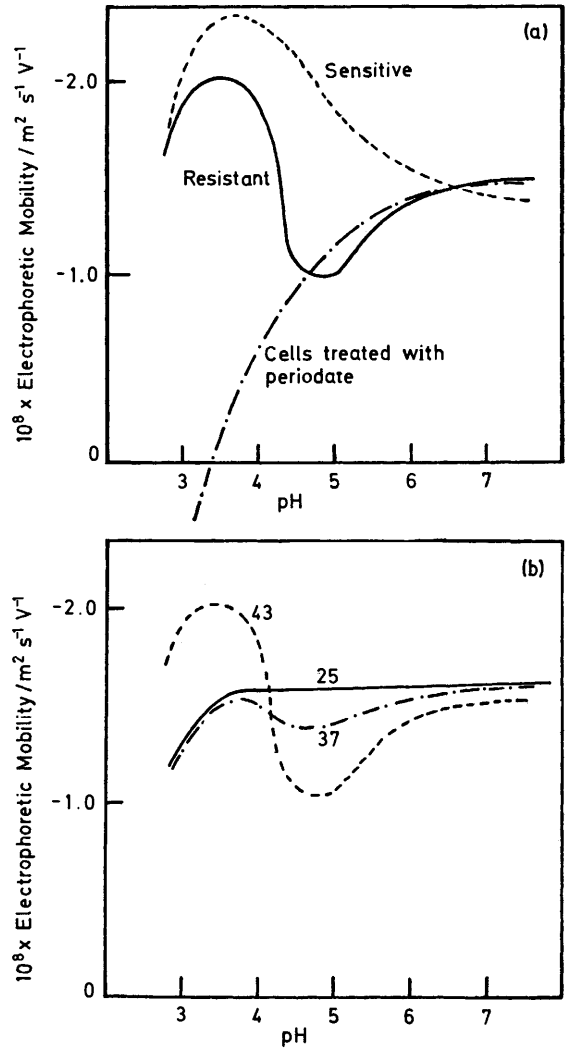

Figure 7 *pH–Mobility curves for cells of* Staph. aureus. *measured in acetate–barbiturate buffer solution.*[32,47] (a) *methicillin-sensitive* (- - - -); *methicillin-resistant* (———) *cells grown at* 37 °C; *oxidized cells* (– · – · – · –); (b) *methicillin-resistant cells grown at* 43 °C (- - - -), 37 °C (– · – · – · – · –), and 25 °C (———)

have lost their ability to produce a β-lactamase; the surface properties are characteristic of sensitive cells.[48] These facts support the hypothesis that the plasmid controlling methicillin resistance also controls the surface properties of

[48] A. M. James and S. M. S. Al-Salihi, *Microbios Letters*, 1976, **1**, 177.

the cells, the surface teichoic acid, and the phosphatase enzyme system[49] in the cells. Irreversible loss of this plasmid during growth at high temperatures results in changes of sensitivity and surface properties of the cells.

Cells of strains of *Pseudomonas aeruginosa* grown at 37 °C can be classified as sensitive or resistant to gentamicin according to the shape and position of the pH–mobility curves.[50] The surfaces of cells of all strains are very complex; the differences between the surfaces of gentamicin-resistant and -sensitive cells are due in part to a different ratio of amino and carboxyl groups. Cells with transferable *R*-factor gentamicin resistance exhibit the surface properties of cells of the gentamicin-sensitive strains,[51] thus making it possible to establish for a resistant strain whether the resistance is due to transmissible *R*-factors.

The Effect of Surface-active Agents in the Suspension Medium. Studies of the variation of the mobility with ionic strength and pH of the suspension medium give no information about the presence of surface lipids, other than phospholipids. Much information has been obtained from a study of the variation of the mobility of organisms in the presence of anionic and cationic wetting agents;[26,30,42,52] lipophilic surfaces exhibit a marked increase of negative charge in the presence of an anionic agent. A cationic agent causes a lowering of the negative charge and charge reversal for a lipid surface; this is partly due to the presence of the large positive ion and partly to the interaction of the agent with the lipid.

Marked changes in the mobility of bacteria from widely different taxonomic groups in the presence of surfactants have been interpreted in terms of the chemical constitution of the surface.[6] Since surfactants are lytic, the effect on the mobility of intact bacteria must be studied at sub-lytic concentrations (for sodium dodecyl sulphate, SDS, this is 10^{-4} mol dm^{-3}). Under these conditions any change in the mobility value can be attributed to the presence of surface lipid and not to the re-adsorption of lysed material. The *S*-value, defined as the relative increase in mobility at pH 7 in the presence of 10^{-4} mol dm^{-3} SDS, expressed as a percentage,[53] is used to indicate the level of surface lipid; an *S*-value > 10 is significant and indicates the presence of surface lipid.

There is no correlation between surface lipid on cells of *Staph. aureus* and their resistance to antibiotics.[53] Cells of *Strep. pyogenes* grown in the presence of glycerol, acetate, or oleate have *S*-values in excess of 30, with a total solvent-extractable cell wall lipid $> 20\%$; in contrast, when the same cells were grown in glucose-broth, the *S*-value was 0 and the total extractable lipid about 1%.[30] The stimulation of lipid production by the different growth conditions resulted in the formation of both neutral and phosphatide lipid; the presence of phosphatide lipid was demonstrated by a change in the position of the pH–mobility curve

[49] A. L. Davies and A. M. James, *Microbios*, 1974, **10**, 257.
[50] D. T. Pechey and A. M. James, *Microbios*, 1974, **10A**, 111.
[51] D. B. Chapman and A. M. James, *Microbios*, 1976, **16**, 111.
[52] A. M. James, 'Surface Activity and the Microbial Cell', S. C. I. Monograph No. 19, 1965, p. 3.
[53] N. J. Marshall and A. M. James, *Microbios*, 1971, **4**, 217.

towards lower pH values. Tetracycline-sensitive *Strep. pyogenes* contained 4—6% (w/w) cell wall lipid and no detectable surface lipid while cells of tetracycline-resistant strains with up to three times the amount of extractable lipid possessed considerable amounts of surface lipid. The surface was not completely covered with lipid; the pH–mobility curves of these cells were still characteristic of a mixed carboxyl–amino surface similar to that for tetracycline-sensitive cells.

Cells of gentamicin-resistant strains of *P. aeruginosa* (MIC >12.5 $\mu g\ cm^{-3}$) have significant amounts of surface lipid; in contrast, cells of sensitive strains have little or no surface lipid. The total solvent-extractable lipid (10% total dry cell weight) is, however, independent of the sensitivity or resistance to gentamicin, suggesting that when the cells acquire resistance there is a redistribution of the lipid so that some is now located at the surface.[54] The information currently available permits no definite conclusion as to whether the presence of surface lipid on naturally occurring resistant cells of *P. aeruginosa* and the changes of the type and position of lipid within the cell are the result of the resistance or whether they are associated properties, originating from a common cause.

B. Blood Cells.—In contrast to bacterial and yeast cells, blood cells do not have a rigid cell wall and are easily lysed in suspensions of low tonicity; the lysate may be adsorbed onto remaining intact cells, thereby altering their surface properties. Such difficulties have been overcome by the use of solutions of high ionic strength, by the inclusion of sorbitol in the suspension medium, and by 'fixing' the cells with aldehydes.

Electrokinetic studies have revealed the polyanionic character of the erythrocyte and the range of conditions of pH and ionic strength under which the surface of the red blood cell is stable.[55] More recently, the relevance of cell charge to aggregation and rouleaux formation has been suggested. The mobility of mammalian erythrocytes varies from one animal species to another; within a species the values are very reproducible.[56,57,58] The mobility of human erythrocytes is independent of the method of blood collection and the age, race, sex, and blood group of the donor, although there is evidence that the mobility decreases with increasing age of the cell. With increasing age of the rat (from foetus to young adult) there is a decrease in the volume of the red blood cells and a concomitant increase in the surface charge density,[59] this is possibly due to structural rearrangement associated with the change in size during maturation of the cell.

Although there are several references in the literature to a change of the mobility of erythrocytes accompanying various diseases[60] (*e.g.* slowing factors in the serum of patients with various carcinomas), very few have been substan-

[54] D. T. Pechey, A. O. P. Yau, and A. M. James, *Microbios*, 1974, **11**, 77.
[55] D. H. Heard and G. V. F. Seaman, *J. Gen. Physiol.*, 1960, **43**, 635.
[56] T. Tenforde, *Adv. Biol. Med. Phys.*, 1970, **13**, 43.
[57] J. N. Mehrishi, *Progr. Biophys. Mol. Biol.*, 1972, **25**, 1.
[58] G. V. F. Seaman, *J. Supramol. Structure*, 1973, **1**, 437.
[59] F. Doljanski and N. Schulman, *Exp. Cell Res.*, 1964, **36**, 605.
[60] A. Rottino and J. Angers, *Cancer Res.*, 1961, **21**, 1445.

tiated.[61] Nevertheless the technique is useful in establishing pathological conditions in man. Each type of cell in the blood of a healthy individual possesses a characteristic mobility which shows only a small scatter; the results, presented as a haemocytopherogram (Figure 8a), show that the erythrocytes, lymphocytes, and

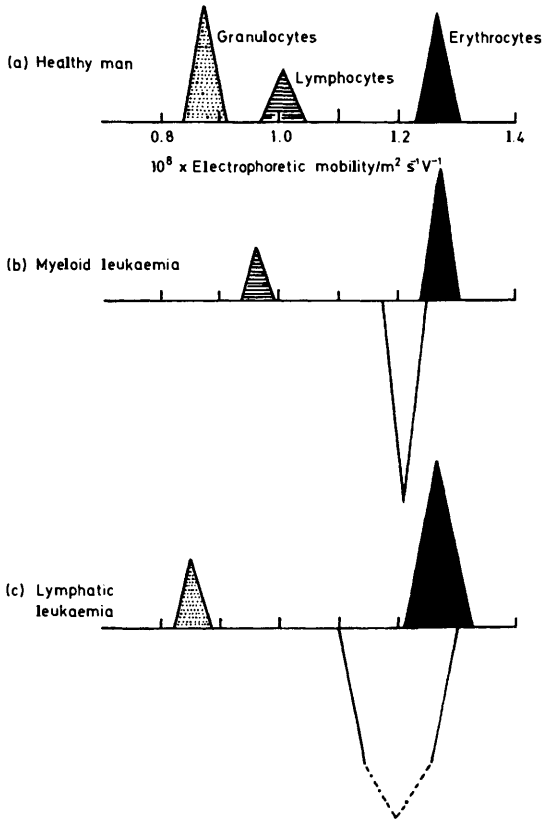

Figure 8 ***Haemocytopherograms of*** (a) ***healthy man***; (b) ***myeloid leukaemia***; ***and*** (c) ***lymphatic leukaemia***[62]

granulocytes form solid groups with standard deviations of about 8%.[62] For samples of blood from leukaemia (myeloid type) patients the normal group of granulocytes, almost missing (Figure 8b), is replaced by a faster moving fraction; the mobility of the erythrocytes and lymphocytes of such patients is unchanged. Similarly, in samples taken from cases of lymphatic leukaemias the lymphocytes are replaced by a fast-moving fraction near the erythrocyte band (Figure 8c); the mobility of the erythrocytes and granulocytes is unchanged.

[61] J. Nadell and W. P. Creger, *Blood*, 1964, **24**, 757.
[62] G. Ruhenstroth-Bauer, in ref. 7, p. 66.

The electrophoretic mobility of red cells of patients with arterial disease is significantly less than in the control patients; the differences are greater in females than in males.[63] There is evidence of a 'slowing factor' in red cells from patients with occlusive arterial disease, suggesting the presence of such a slowing factor in the plasma of such patients. This factor, by facilitating red cell aggregation, may play a part in the pathogenesis of arterial disease. The surface properties of promenisococytes, sickle cells, and normal human erythrocytes do not differ significantly from one another, and no detectable differences have been observed between normal erythrocytes and sickle cells with regard to the adsorption of haemoglobin and serum proteins.[64]

The range of ionic strength and pH of the suspension medium under which human erythrocytes show reversible or partially reversible electrokinetic behaviour has been established.[55] The decrease in stability at ionic strengths less than physiological (Figure 9) is due to the effects of increased leakage of haemo-

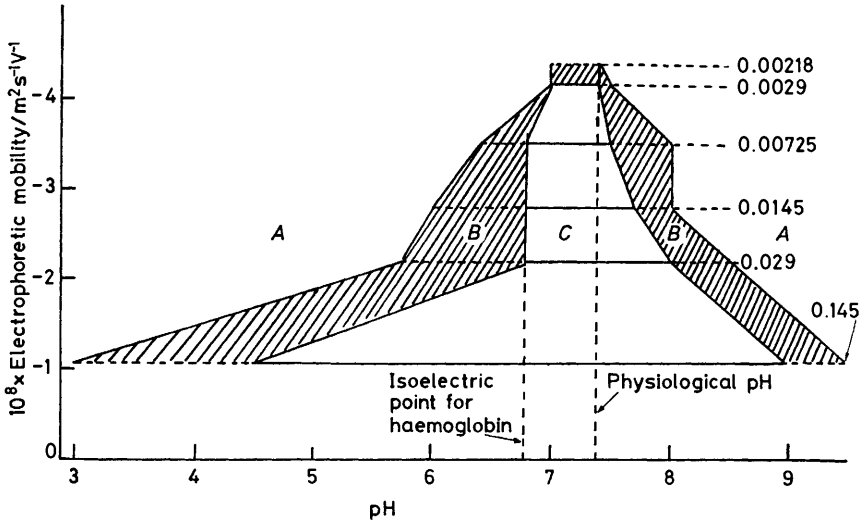

Figure 9 *Variation of the electrokinetic stability of the normal human erythrocyte with pH and ionic strength* (I/mol dm^{-3});[7,55] *A: region of instability; B: region of metastability; and C: region of stability*

globin coupled with its ready adsorption onto the cells, especially in suspension at low pH values. Under conditions outside the range of stability, irreversible changes in the surface properties result from the irreversible adsorption of the products of haemolysis. The stability of red blood cells to suspension under non-physiological conditions can be greatly increased by 'fixation' with aldehydes.[65]

[63] T. B. Begg, I. M. Wade, and B. Bronte-Stewart, *J. Atheroscler. Res.*, 1966, **6**, 303.
[64] G. V. F. Seaman and B. A. Pethica, *Biochem. J.*, 1964, **90**, 573.
[65] D. H. Heard and G. V. F. Seaman, *Biochim. Biophys. Acta*, 1961, **53**, 366.

Caution must be exercised in the interpretation of electrokinetic data of cells after treatment with formaldehyde or acetaldehyde, since leakage of intra-cellular proteins followed by adsorption onto the surface of the cells may occur.[66]

In suspension in standard saline or phosphate buffer solution, human erythrocytes exhibit a very reproducible mobility.[55,67] The lack of specificity towards anions is indicative of the absence of any significant number of surface positive groups. This is further confirmed by the constancy of mobility in the pH range 7—10 and the lack of change of mobility of the cells after treatment with acetaldehyde, 1-fluoro-2,4-dinitrobenzene, and *p*-tosyl chloride. It has been conclude that the human erythrocyte is a macropolyanion (number of negative surface groups *ca.* 10^7 per cell; number of positive groups $< 2 \times 10^5$ per cell). The surface charge arises solely from esterifiable anionic surface groups; the effects of ion adsorption at the plane of shear are negligible. The pK of the surface groups of normal erythrocytes (I = 0.145 mol dm^{-3}) is about 2.5 and of aldehyde-fixed cells 2.6—2.8. Treatment of native or aldehyde-fixed erythrocytes with neuraminidase results in the release of almost all of the membrane-bound sialic acid (pK 2.6) and a marked decrease in the mobility of the cell[68] (Figure 10). Thus the negative charge of human erythrocytes and indeed the erythrocytes of many species[69,70] is due mainly to the carboxyl group of a sialic acid rather than, as previously thought, to the phosphate groups of phospholipids, *etc.* *N*-Acetylneuraminic acid constitutes 61.5% of the anionic groups of the intact erythrocyte; when these have been eliminated by treatment with neuraminidase, the remaining 38.5% have a pK of 3.35, possibly due to the α-carboxyl groups of polypeptides or proteins. The quantity of sialic acid released is about twice that calculated from the change in the mobility, if it is assumed that equation (5) is applicable and that the carboxyl group of every sialic acid molecule is fully effective at the electrokinetic surface. Since such a calculation can be in error by a factor of up to 2 [the α-factor of equation (5a)], the difference between the amount of sialic acid estimated by chemical assay and computed from mobility data is probably not significant. Treatment of erythrocytes with proteolytic enzymes (trypsin, ficin, pronase, *etc.*) releases sialic acid in bound form;[70] this is usually accompanied by a decrease in the mobility of the cells. Such changes must be interpreted with care since these could have arisen not only from the specific action of the enzyme but also from other enzyme impurities, from the adsorption of the enzyme or its decomposition products, from the adsorption of material derived from intra-cellular leakage, or even from reorientation of ionogenic groups. Table 2 summarizes the results of experimental work on human erythrocytes and other blood cells. Oligosaccharide chains at the surface of red blood cells contain about five molecules of neutral sugars (glucose, galactose, *N*-acetylglucosamine, and *N*-acetylgalactosamine).

[66] P. S. Vassar, J. M. Hards, D. E. Brooks, B. Hagenberger, and G. V. F. Seaman, *J. Cell Biol.*, 1972, **53**, 809.
[67] G. V. F. Seaman and D. H. Heard, *J. Gen. Physiol.*, 1960, **44**, 251.
[68] G. M. W. Cook, D. H. Heard, and G. V. F. Seaman, *Nature*, 1961, **191**, 44.
[69] E. H. Eylar, M. A. Madoff, O. V. Brody, and L. J. Oncley, *J. Biol. Chem.*, 1962, **237**, 1992.
[70] G. V. F. Seaman and G. Uhlenbruck, *Arch. Biochem. Biophys.*, 1963, **100**, 493.

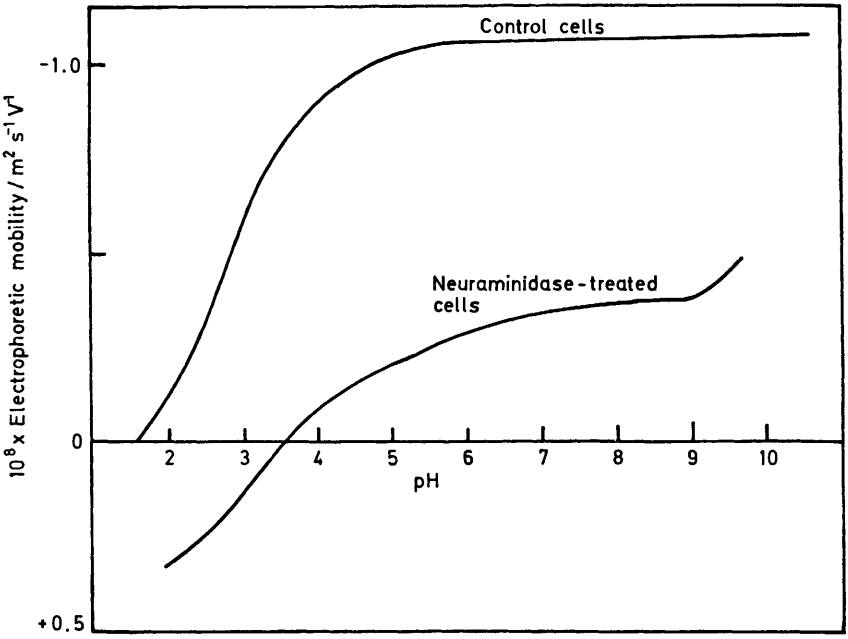

Figure 10 *pH–Mobility curves for human erythrocytes before and after treatment with neuraminidase; I* = 0.145 mol dm^{-3}

Other blood cells cannot be classified as macropolyanions. In addition to sialic acid residues, phosphate, amino, and sulphydryl charged groups have been detected by elimination, masking, and exposure on treatment with specific enzymes or by chemical blocking agents.[57] Thus treatment with neuraminidase has been used to demonstrate the presence of α-carboxyl groups of *N*-acetylneuraminic acid on the surface of human blood platelets,[71] of human lymphocytes,[72] and of human leucocytes.[73] Ribonuclease-sensitive phosphate groups are present on human lymphocytes and some tumour cells[74] and alkaline-phosphatase-sensitive phosphate groups on human blood platelets.[75] Chemical blocking agents have been used to demonstrate the presence of positively charged amino-groups[76] and sulphydryl groups[77] on human lymphocytes and platelets.

If assumptions are made about the value of the permittivity and coefficient of viscosity within the electrical double layer and also the validity of equations (3), (4), and (5) for penetrable surfaces, then the contribution of each type of group to

[71] G. V. F. Seaman and P. S. Vassar, *Arch. Biochem. Biophys.*, 1966, **117**, 10.
[72] J. N. Mehrishi and A. E. R. Thomson, *Nature*, 1968, **219**, 1080.
[73] P. S. Vassar, M. J. Kendall, and G. V. F. Seaman, *Arch. Biochem. Biophys.*, 1969, **135**, 350.
[74] E. Mayhew and L. Weiss, *Exp. Cell Res.*, 1968, **50**, 441.
[75] J. N. Mehrishi, *Nature*, 1970, **226**, 452.
[76] J. N. Mehrishi, *European J. Cancer*, 1970, **6**, 127.
[77] J. N. Mehrishi and D. R. Grassetti, *Nature*, 1969, **224**, 563.

the total surface charge can be calculated and, from a knowledge of the dimensions of the cell, the number of groups on each cell can be estimated (Table 2). Although there is very little difference between the mobility values of the various cells, there is a marked difference in the total number of groups per cell, due to the different surface areas. Two types of packing for the groups in clusters (hexagonal and cubic) on the surface of platelets, lymphocytes, and Ehrlich tumour cells have been proposed;[57] so far there is no evidence to support either model.

The calcium-binding sites of human erythrocytes and leucocytes comprise the neuraminate ions and, at present, unidentified sites, some with greater affinity than the neuraminate ions and some with little or no affinity;[78] it was concluded that calcium ions would form poor ligands for direct intercellular binding. Platelet aggregation by bivalent ions, a temperature-dependent reversible physical chemical process, results from the interaction with platelet phosphate groups.[57]

The lymphocyte population in mammals consists of two main classes: thymus-derived (T) and non-thymus or bone-marrow-derived (B) lymphocytes, which are distinguished by their different surface antigenic characteristics, by the presence of large amounts of surface associated immunoglobins (sIg) on B cells and their absence on T cells, and by their property of forming different types of rosettes. The T and B cells in the mouse exhibit different mobility values; T cells have a significantly higher charge than do B cells.[79] Human lymphocytes exhibit a bimodal population; the major part of the population, with the higher charge, represent the T cells and the remainder, of lower charge, represent the B cells. A similar dichotomy occurs with lymphocytes from human tonsils;[80] 61% of the population, B cells, have a mobility of 0.81 and 39% of the population, T cells, have a mobility of 1.06×10^{-8} m^2 s^{-1} V^{-1}. The percentage of sIg cells as determined by immunofluorescence, however, is always less than that of the slow-moving B cells. While the mobility values of the B cells in tonsils and blood are very close, those of the T cells differ markedly; T cells in tonsils have a lower mobility than do those in blood (1.20×10^{-8} m^2 s^{-1} V^{-1}). This difference may be attributed to the fact that the infected tonsils, already engaged in an immune response against infecting bacteria, have lost their capacity for response to another antigen.

There is a significant decrease in the total population of fast-moving T lymphocytes in solid tumour subjects, especially in metastatic patients; this is in agreement with the variation of T lymphocyte population observed in cancer patients by other methods.[81] The observed changes in mobility could be indicative of selective alterations of the cell surface, as a result of metabolic changes, in some cell sub-population. In patients with rheumatoid arthritis the mean

[78] G. V. F. Seaman, P. S. Vassar, and M. J. Kendall, *Arch. Biochem. Biophys*, 1969, **135**, 356.
[79] D. Sabolovic, N. Sabolovic, and F. Dumont, *Lancet*, 1972, **ii**, 927.
[80] F. Dumont, *Biomed.*, 1974, **21**, 17.
[81] R. Plagne, Ph. Chollet, D. Guerin, J. Chassagne, J. M. Bidet, and B. Sauvezie, *Biomed. Express*, 1975, **23**, 447.

Table 2 *The topochemistry of various blood and tumour cells*

Cell type	*Cell area* /μm^2	$10^8 \times$ *Mobility**/ $m^2 s^{-1} V^{-1}$	$10^2 \times$ *Charge density* /$C m^{-2}$	$10^{-5} \times$ *charges (or groups) per cell surface area*					
				Acidic groups		*Phosphate groups*		*Amino*	*Sulphydryl*
				NANA	*Unidentif.*	*RNase*	*Alkaline phosphatase*		
Human erythrocyte	163	1.08	1.22	62.0	40.9	—	—	n.d.	n.d.
Human lymphocyte	113	1.09	1.23	29	55.5	8.7	—	9.5	19.8
Human platelet	28	0.85	0.96	8.9	6.5	—	5.0	2.4	2.8
Ehrlich ascites tumour	900	1.08	1.22	235	391	119	—	117	361
Mouse B lymphocyte	100	0.90	1.02	1.2	4.3	1.6	—	1.1	19.6
Mouse T lymphocyte	100	1.33	1.52	2.4	7.6	n.d.	—	0.6	n.d.

* Mobility measured in 0.145 mol dm^{-3} NaCl at pH 7.2, data from refs. 57 and 83

mobility of the T cell population of peripheral-blood leucocytes is similar to that of the control; however, the mean mobility of the slow-cell population is significantly less than that of the control.[82] Additionally, the charge carried by the polymorphonuclear cell population in patients with rheumatoid arthritis is significantly less than that in the blood of controls. The alteration of the surface properties of the leucocytes could be due to impaired maturation, to an unusual response to a stimulus, or to the influence of an external agent which may itself be electrically charged. Bound antibodies could mask charged groups on the surface, while viruses could modify cell surface components. This modification of cell topochemistry may thus be associated with abnormal cell function.

There are marked differences in the surface properties of mouse T and B lymphocytes;[83] the B cells carry negatively charged sialic acid moieties, unidentified acidic groups and phosphate, positively charged amino groups, and sulphydryl groups (Table 2). The more negatively charged T cells have no surface phosphate or sulphydryl groups. These differences in surface properties cannot satisfactorily be explained by the presence of sIg on the surface of B cells and its absence on T cells.

C. Tissue and Tumour Cells.—Many properties of mammalian cells are expressed at, or mediated by, the cell surface, and among these properties are those which distinguish cancer from normal cells. Many of the destructive properties of tumour cells, *e.g.* local invasion, metastasis, and loss of contact inhibition, are believed to depend on changes in the nature of the tumour cell membrane.[84] Tumour cells have a high negative surface charge density, and the decrease in mutual adhesiveness is probably a reflection of changes in the surface components following malignant transformation. While it is likely that there is a direct correlation between surface sialic acids and surface charge, the assumption that tumour cells (or any other type of cell) repel one another because their surfaces are highly negatively charged, thus rendering them non-adhesive, is now admitted to be too simple. There has been no adequate demonstration of a correlation between surface charge and lack of tumour cell adhesiveness. On the contrary, there are serious objections to the possibility that the adhesion of tissue cells takes place solely on the interplay of physical forces. Since polyornithine and polylysine possess antitumour activity and also change the surface charge to zero or positive values, it has been suggested that the negative charge of the tumour cell surface may well play a role in its highly invasive properties.[85]

Each tumour has a characteristic histogram of distribution of mobility values which does not change during years of continued transplantation.[86] The charge density of kidney tumour cells is almost double that of the normal homologous cells; in rat hepatoma the observed increase was 31%. This increased surface

[82] K. A. Brown, E. J. Holoborow, and A. J. Collins, *Lancet*, 1977, **i**, 114.
[83] J. N. Mehrishi and K. Zeiller, *European J. Immunol.*, 1974, **4**, 474.
[84] P. Emmelot, *European J. Cancer*, 1973, **9**, 319.
[85] J. N. Mehrishi, *European J. Cancer*, 1969, **5**, 427.
[86] J. H. B. Lowick, L. Purdom, A. M. James, and E. J. Ambrose, *J. Roy. Microscop. Soc.*, 1961, **80**, 47.

charge density *per se* is not a specific manifestation of malignancy since it also occurs in the course of regulated growth processes.[87] The underlying mechanism producing the changes accompanying normal restorative growth may differ from that associated with malignant transformation. The higher mobility of embryonic mouse fibroblasts as compared with that of adult fibroblasts supports this view.

Variations in the mobility of Ehrlich ascites cells extracted from tumours of the same age have been reported; these variations are much greater than those found for erythrocytes and arise after injection of cells from a common population. During growth *in vivo* the mean mobility reaches a maximum value one to two days after tumour inoculation and then declines until the death of the animal.[88] Cells cultured *in vitro* exhibit a low mean mobility which is only attained after 14—16 days' growth in mice.

Neuraminidase-susceptible sialic acid has been detected on the cell surface;[89,90] besides being involved in genetic systems, sialic acid contributes to the bulk of the negative charge of the cells and also masks tumour antigens. In addition, alkaline-phosphatase-susceptible phosphate groups and unidentified anionic,[75] amino,[75] and sulphydryl[77] groups have been identified on Ehrlich ascites cells. On account of the large surface area of these cells, the numbers of surface groups are very much greater than those on blood cells (Table 2). The interaction between amino and sulphydryl groups and the components of tobacco smoke, such as aldehydes and free radicals, is of clinical interest and is worthy of study, possibly by e.s.r. and n.m.r. spectroscopy.

The distribution of positively charged colloidal iron(III) hydroxide particles on glutaraldehyde-fixed Ehrlich ascites cells suggests the presence of different sized neuraminidase- and ribonuclease-susceptible zones at the cell surface; these have a higher than average density of anionic sites.[91] The problems associated with fixation and other artefacts pose considerable difficulties and prevent the extrapolation of these results to living cells.

The macrophage electrophoretic mobility (MEM) test, an *in vitro* blood test for cancer, depends on the sensitization of the patient's lymphocytes to a common antigen present in human tumours.[92] Peripheral lymphocytes from patients with malignant disease sensitized to a basic protein derived from human brain release a substance, known as the macrophage slowing factor, which reduces the mobility of macrophages isolated from the peritoneal exudate of albino guinea-pigs. Patients with malignant disease produce a slowing of macrophage of between 13 and 30%; in contrast, the slowing in healthy controls is less than 4%; this emphasizes the absence of overlap between malignant and normal subjects. This technique has been extended to a study of the assessment of the compatibility between live donor/recipient pairs and cadaver donor/recipient pairs for trans-

[87] S. Eisenberg, S. Ben-Or, and F. Doljanski, *Exp. Cell Res.*, 1962, **26**, 451.
[88] E. Mayhew, *Cancer Res.*, 1968, **28**, 1590.
[89] P. D. Ward and E. J. Ambrose, *Cell Sci.*, 1969, **4**, 289.
[90] D. Patinkin, M. Schlesinger, and F. Doljanski, *Cancer Res.*, 1970, **30**, 489.
[91] L. Weiss, O. S. Jung, and R. Zeigel, *Internat. J. Cancer*, 1972, **9**, 48.
[92] B. K. Shenton and E. J. Field, *J. Immunol. Methods*, 1975, **7**, 149.

plantation.[93] The test measurement of lymphocytic interaction is significantly correlated with histocompatibility as measured by serotyping. It has advantages in the field of histocompatibility assessment as there is no reference to an individual antigen and the test can be performed in a very short time. Lymphocytes from patients with multiple sclerosis are more susceptible to the inhibitory activity of linoleic acid when tested by the macrophage electrophoretic mobility test for sensitization to thyroid than are cells from normal subjects or those with a variety of other neurological diseases. These differences, which can be used as a specific diagnostic test for multiple sclerosis, offer a rational basis for the observation that the supplementation of the diet by sun-flower seed oil (rich in linoleic acid) has a beneficial effect on the course of multiple sclerosis.[94]

D. Other Cells.—*Fungal Cells.* Many air-borne fungal spores have a water-repellant surface which aids dispersal, prevents desiccation, and may provide a barrier to the entry of toxic agents. The surface lipid on *Neurospora crassa*, *Alternaria tenuis*, and *Botrytis fabae*[95,96] and on actinomycete spores[97] is probably sufficient to account for the hydrophobic properties and water-repellant properties of these cells. The surfaces of spores of different species are constant and species-specific when grown under defined conditions. *Penicillium cyclopium* and *P. spinulosum* have a wax or lipid surface, with possibly a polysaccharide component in the case of the latter; *Fusarium lini* and *Mucor ramannianus* have an acidic polysaccharide; *N. crassa* has amino, carboxyl, and phosphate groups but the phosphate groups are absent from washed spore walls; the conidia of *P. expansum* are covered with a phosphate layer which is easily washed off, revealing a mixed amino–carboxyl surface.[98] Protoplasts isolated from various mycelia and conidia have a predominantly protein surface. Dodine, a cationic fungicide, binds to the spore surface of *N. crassa* and reduces the charge to zero at a lower concentration than that required to kill the spores, thus detoxifying the fungicide.[96]

Yeasts. Cells of *Saccharomyces cerevisiae* and *C. carlsbergensis* have a mixed surface with amino, carboxyl, and phosphate groups;[99] one strain produced a phosphate-free surface when grown in the absence of phosphate. Lipid is not a surface component of *S. cerevisiae*.[100] Surface charged groups play no direct part in the flocculation of yeast cells.[95]

Chlorella. During the transformation of the D-form of *Chlorella* cells into L-stage cells in the light, the negative value of the mobility decreases and, during

[93] B. K. Shenton, P. R. Uldall, J. Swinney, and E. J. Field, *Tissue Antigens*, 1975, **5**, 246.
[94] E. J. Field, B. K. Shenton, and G. Joyce, *Brit. Med. J.*, 1974, **i**, 412.
[95] D. V. Richmond and D. J. Fisher, *Adv. Microbial Physiol.*, 1973, 9, 1.
[96] E. Somers and D. J. Fisher, *J. Gen. Microbiol.*, 1967, **48**, 147.
[97] H. W. Douglas, S. M. Ruddick, and S. T. Williams, *J. Gen. Microbiol.*, 1970, **63**, 289.
[98] D. J. Fisher and D. V. Richmond, *J. Gen. Microbiol.*, 1969, **57**, 51.
[99] A. A. Eddy and A. D. Rudin, *Proc. Roy. Soc.*, 1958, **B148**, 419.
[100] M. S. Briley, R. F. Illingworth, A. H. Rose, and D. J. Fisher, *J. Bacteriol.*, 1970, **104**, 588.

the subsequent period of darkness, as the cells divide, the mobility increases to a value characteristic of the D-form. These changes during growth in the light are attributed to developmental changes in the cell surface.[101]

Spermatozoa. In suspension, at pH values in excess of the isoelectric point (pH 3.4), immobile bull and rabbit spermatozoa migrate towards the positive electrode with the tail extended forward; below pH 3.4, migration is towards the negative electrode with the head in the lead (*i.e.* the same head-to-tail orientation with respect to the electric field). Mobile spermatozoa exhibit galvanotactic migration towards the negative electrode, *i.e.* in the opposite direction to electrophoretic migration.[102] There is little evidence of difference of charge on male- or female-determining spermatozoa or support for the claims of electrophoretic separation of the two types.[103]

Mitochondria. The surfaces of mitochondria from rat kidney and liver[104] and from pea and cauliflower[105] carry different amounts of similar ionogenic groups and, in spite of the heterogeneous nature of the tissue, the population of mitochondria from each tissue is electrokinetically homogeneous.

6 Conclusions

The study of the electrical properties of particles in suspension reveals important information concerning the structure of the surface layers of non-ionogenic particles and the charge-determining groups on biological cell surfaces.

Care must be exercised in the preparation of particles or cells with a clean, uncontaminated, and reproducible surface and in the experimental techniques for measurement before the experimental data can be interpreted with confidence.

Further studies of the interaction and/or adsorption of various species at non-ionogenic surfaces will increase our understanding of the processes which occur at such surfaces. The interpretation of such data will be facilitated by complementary studies using high-resolution stereoscan electron microscopy.

More detailed information on the cell surface molecular complex of biological cells will provide a basis for a better understanding of the mechanism of cell adhesion, platelet aggregation, antigen–antibody, bacteria–virus, and bacteria–drug interactions, *etc.*

[101] S. Lukiewicz and W. Korohoda, in ref. 7, p. 171.
[102] A. C. Nevo, I. Michaeli, and H. Schindler, *Exp. Cell Res.*, 1961, **23**, 69.
[103] A. D. Bangham, *Proc. Roy. Soc.*, 1961, **B155**, 292.
[104] D. T. Plummer, *Biochem. J.*, 1964, **93**, 9P.
[105] H. W. Douglas, M. V. Laycock, and D. Boulter, *J. Exp. Bot.*, 1963, **14**, 198.