PROGRESS IN BIOPHYSICS

AND

MOLECULAR BIOLOGY

VOLUME 25

PROGRESS IN BIOPHYSICS
AND MOLECULAR BIOLOGY
25

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PERGAMON PRESS
OXFORD · NEW YORK · TORONTO
SYDNEY · BRAUNSCHWEIG

Pergamon Press Ltd., Headington Hill Hall, Oxford
Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523
Pergamon of Canada Ltd., 207 Queen's Quay West, Toronto 1
Pergamon Press (Aust.) Pty. Ltd., 19a Boundary Street, Rushcutters Bay, N.S.W. 2011, Australia
Vieweg & Sohn GmbH, Burgplatz 1, Braunschweig

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First edition 1972

Library of Congress Catalog Card No. 50–11295

Printed in Great Britain by A. Wheaton & Co., Exeter

08 016935 X

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MOLECULAR ASPECTS OF THE MAMMALIAN CELL SURFACE

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CONTENTS

1

MOLECULAR ASPECTS OF THE MAMMALIAN CELL SURFACE

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I. INTRODUCTION

All living matter is surrounded by membrane. Considering their biological importance, remarkably little is known about the protein composition of membranes, the structure of which is of interest in several fields of research. Many reactions of considerable interest in biology and medicine (in immunity and disease) involve the cell surface. Obviously, the surface of cells is more easily accessible to pharmacological agents than the inside of the cells.

It is vital to determine the precise chemical nature of the cell surface molecular components, their distribution, and the stereochemical arrangements of the groups which participate in reactions at the cell surface.

The knowledge about the cell surface structures at the molecular level should clearly prove to be of some practical value in the development of suitable therapeutic agents. The gaining of such knowledge about the cell peripheral regions is one of the most important aspects of cell membrane studies. The aim of these studies is also to relate the behaviour of cells and the various cellular interactions to the molecular constitution of cell surfaces. A considerable amount of work is now being carried out on the various aspects of cell membranes, and the information continues to accumulate rapidly. Several monographs, review articles, and many papers relating to the cell surface have been published (Abramson, 1934; Abramson, Moyer, and Gorin, 1942; Bier, 1959; James, 1957; Abercrombie and Ambrose, 1962; Curtis, 1967; Malhotra, 1970; Mehrishi, 1971a,b, 1972a,b; Vassar, Seaman, and Brooks, 1967; Weiss, 1967, 1969).

Review of all aspects of the cell surface needs considerable space and warrants writing a book. I have therefore selected topics which have interested me because of their relevance to my own work. The personal selection of some other subject-matter is based on thoughts for future research and is prompted by a suspected lack of general awareness. It is proposed to describe some of the recent developments in the biophysical and biochemical methods and to demonstrate their usefulness for obtaining information about the cell periphery.

Relevant studies on human blood platelets, leucocytes, lymphocytes, Ehrlich ascites tumour cells, BP8 ascites tumour cells, thymocytes, lymph node cells, and macrophages will be discussed. The biological implications of the cell surface molecular components in some biological phenomena will be considered.

II. THE ELECTRIC CHARGE AND CELL ELECTROPHORESIS

Traced to their very sources, all forces—chemical, colloid–chemical, and physical—are electrical in nature. For example, the four types of ionizing systems in equilibria may be considered.

Typical ionogenic systems

$$\begin{aligned} -COO^- &+ H^+ \rightleftharpoons -COOH \\ -NH_2 &+ H^+ \rightleftharpoons -NH_3^+ \\ -OSO_2O^- &+ H^+ \rightleftharpoons -OSO_2H \\ -OPO_3H^- &+ H^+ \rightleftharpoons -OPO_3H_2 \end{aligned}$$

The chief problem of colloid chemistry and chemistry of the cell surface is the investigation of the structure of the components which determine its electrical behaviour.

The acidic and basic amino acids are largely ionized in aqueous electrolytic media. The charge of the carboxyl group and α-amino group disappear during the formation of peptide bonds. However, proteins exhibit an amphoteric nature, acidic groups in the side chains of glutamyl and aspartyl residues are dissociated at physiological pH and bear negative charges, and the basic groups of lysine and arginine (and to a lesser degree histidine) bear positive charges. The degree of ionization of the groups depends upon the pH of the solution. An excess of H^+ ions, i.e. low pH, retards the dissociation of the acidic groups; at high pH the basic groups remain uncharged. Clearly, when the positive and the negative charges at a particular pH are equal, the net charge is zero, and this point is called the *isoelectric point* (*i.e.p.* or *pI*). There is no movement of the protein at this point under the influence of an applied electric field. On the acid side of this point, the protein migrates towards the cathode. If the pH exceeds the i.e.p., then the molecules are negatively charged and migrate towards the anode.

Let us consider the ionization of an acid HA to give H^+ and A^- ions in equilibrium with HA according to the following set of equations:

$$H^+ + A^- = HA$$

$$\frac{[H^+]\,[A^-]}{[HA]} = K$$

or

$$\frac{1}{[H^+]} = \frac{[A^-]}{K[HA]},$$

hence

$$pH = \log_{10}\frac{1}{[H^+]} = \log_{10}\frac{1}{K} + \log_{10}\frac{[salt]}{[acid]}.$$

The expression $\log_{10}(1/K)$ is known as the pK of say the acid HA. $pH = pK + \log\dfrac{[salt]}{[acid]}$ is called the Henderson equation.

The pK is a constant for a given ionic strength, characteristic for a particular ionogenic group, but is modified by neighbouring group effects. One effect is that most pK values are raised as those of the amino and —SH groups as in the case of cysteine (Benesch and

Benesch, 1955). In practical terms the pK of a group may be defined as that pH at which the ionization of the group is one-half. If an ionization curve is followed over a range of pH, the pK characteristic for the group may be determined experimentally.

A. *The Electrokinetic Phenomena*

Two phenomena, closely connected with adsorption, electro-endosmosis and cataphoresis (electrophoresis), were discovered by the Russian physicist Reuss, and first described in 1809.

Cataphoresis may imply migration of particles towards only one electrode, and may be confusing. Therefore the term electrophoresis will be used throughout.

Cells or particles when suspended in an electrolytic medium such as physiological saline acquire an electric charge either by the ionization of the group(s) present on the cell surface or by the redistribution of ions at the particle/medium interface. When the cells are in suspension ions opposite in sign to the charges on the cell surface—called the counter-ions—crowd around the cell surface charges and form the so-called *electrical double layer.* The concentration of these counterions decreases (exponentially) as the distance from the cell surface into the bulk of the liquid decreases. The cell, together with the counterions in the double layer, form one whole kinetic unit, and if the cells in suspension are free to move, they will migrate under the influence of an applied electric field. This phenomenon is termed *electrophoresis.* The potential that the surface charge gives rise to is called the *electrokinetic potential or the zeta potential.*

B. *Mathematical Relations for the Calculation of the Charge*

The calculation of the zeta potential from the measurement of the electrophoretic mobility requires a theoretical relation between the two quantities. The oldest of this kind, which was derived by Helmholtz (1879) and improved by von Smoluchowski (1903), reads:

$$\zeta = \frac{4\pi\eta}{D} u, \tag{1}$$

where η and D are the bulk viscosity and dielectric constant respectively of the suspending medium, and the electrophoretic mobility u is expressed as μm/sec/V/cm. For aqueous solutions of NaCl at 25°C [eqn. (1)] reduces to $\zeta = 12.85\, u$.

For 1–1 electrolytes such NaCl and zeta potentials (ζ) up to 25 mV, the surface charge density σ can be evaluated by a modified form of the Gouy–Chapman equation (Haydon, 1961) set out in a format suitable for computer programming (Mehrishi, 1969a).

$$\sigma = [1 + (1 - \alpha)^{\frac{1}{2}}] \left(\frac{NDkT}{2000\,\pi}\right)^{\frac{1}{2}} I^{\frac{1}{2}} \times 2 \left[\sinh \left[\frac{e}{2kT} \left\{ \frac{4\pi\eta}{D} \left(\frac{\mu\text{m}}{\text{V/cm}} \frac{1}{S} \right) \right\} \right] \right]. \tag{2}$$

For aq. NaCl ($I = 0.145$) at 25°C, this equation becomes

$$\sigma = [1 + (1-\alpha)^{\frac{1}{2}}]\ 13{,}410 \sinh (\zeta/51.3) \tag{3}$$

for zeta potentials up to 25 mV, where N is the Avogadro number (6.024×10^{23} molecules per mole), D the dielectric constant, k the Boltzmann constant (1.3803×10^{-16} erg/deg-molecule), S the observed migration time (sec), T the absolute temperature [$273.15 + t°$ (centigrade)], e the electronic charge (4.802×10^{-10} e.s.u.), I the ionic strength ($\frac{1}{2} \Sigma\, c_i z_i^2$), c the ionic strength of the ions, z the valency of the ions, and α is the fraction of the total space within the surface which is not available to counter ions. It is assumed that σ does not vary within the distance from the surface and that only uni-univalent electrolyte is present.

It should be noted that the surface charge density evaluated from eqn. (3), for the value of $\alpha = 1$, is the same as without the correction factor α which takes into account the penetrable peripheral region of a cell (Haydon, 1961). For surfaces that are penetrable, there will be free ions both inside and outside the surface, and the electrokinetic charge density may be higher by a factor of up to 2 than that calculated with the original Gouy–Chapman equation. The surface charge density computations for the various values of $\alpha = 0, 0.25, 0.5, 0.75$, and 1 using eqn. (3) are tedious and time-consuming. Autocode programmes were written incorporating eqns. (2) and (3) and the apparatus constants and a digital computer (Titan) used routinely for accuracy, speed, and convenience (Mehrishi, 1969a).

The electrophoretic mobility is governed by the pH and the ionic strength [see eqn. (2)] and is independent of temperature (Mehrishi and Seaman, 1966). Fuller accounts about the electrokinetic phenomena and associated phenomena can be found in the various monographs (Bier, 1959; see also Abramson, 1934; Shaw, 1969).

For rapid handling of a great amount of data on cells which show a large variation in the various parameters I have used a digital computer routinely. I have found the use of the computer efficient, convenient, and time saving. Programmes in Autocode and Fortran 4 for the Cambridge University computers Titan and Atlas have been written incorporating the apparatus constants such as the size of the graticule (in microns, μm) mounted in the ocular of the electrophoresis microscope, the length of the electrical path in the electrophoresis cell (in cm), the applied voltage (V in volts), and the other constants of eqns. (2) and (3). The observed times of migration of cells (in sec) following the application of the field strength (X = V/cm) are fed into the computer which processes the data to print out the values of the electrophoretic mobilities (u in μm/sec/V/cm), zeta potential (ζ in mV) [eqn. (1)], and electrokinetic charge densities (e in e.s.u./cm^2) for the individual cells evaluated by eqn. (3) for various values of α ranging from 0 to 1. For the present work an electrophoresis chamber of a circular cross-section has been used and the experiments carried out at constant voltage. For calculation of the electrokinetic parameters from observations of the times of migration in an electrophoresis chamber of a rectangular cross-section at constant current, programmes are easily edited to incorporate the specific resistance of the electrolytic medium, the current, etc. The second programme, which incorporates the standard statistical formulae, processes the individual values of u, ζ, and σ to print out the mean values together with the standard deviations and standard error of the mean. The third programme processes the data to plot histograms from the individual values. The computer-processed histogram plot for the surface area of the Ehrlich ascites tumour cells is reproduced in Fig. 1. When required, the processed data can be run through paired or unpaired t-tests and the computer prints out the degrees of freedom, variances, t-values, and the results of the tests of significance at 0.1, 1, and 5% levels. Appropriate tables are incorporated in the programmes.

Information about the surface regions of cells and particulate matter can be obtained by microelectrophoresis which for clarity will be termed cell electrophoresis, meaning the electrophoresis of single cells in suspension. Since the pioneering work of Abramson and others on the surface properties of many cell types, cell electrophoresis has proved to be a powerful tool for the study of cell surface membranes. The cells in suspension can be examined under strictly physiological conditions without permanent damage to the cell membrane of the intact living cell provided that the measurements are completed without too much delay or harsh handling of the cells. However, it must be emphasized that the technique of cell electrophoresis cannot stand *alone* and needs to be used in conjunction with biochemical methods.

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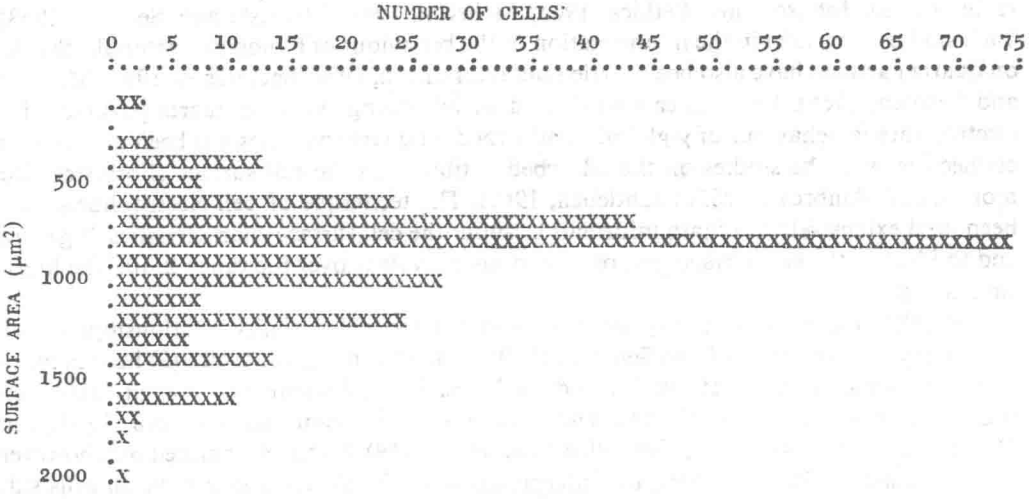

FIG. 1. Computer print-out of the histogram showing the variation of the EAT cell size in terms of μm^2 of the cell surface area. The EAT cell is assumed to be a sphere. Diameters were determined by the hanging drop method. (From Mehrishi, 1969a).

C. *The Sensitivity of the Cell Electrophoretic Technique*

The technique gives information about the net electrokinetic charge and gross changes in the electrokinetic profile. The method is not quite so crude as is perhaps generally suspected. As the following simple calculation will show, 10^6 electron charges ($\pm 5\%$) can be estimated on live intact cells fairly rapidly without resort to highly complicated and sometimes prohibitively expensive apparatus.

The electrophoretic mobility of saline washed human erythrocytes in saline is -1.08 ± 0.02 μm/sec/V/cm (Seaman and Heard, 1960), and this corresponds to about 10^7 electron charges. The bulk of the surface charge is attributed to the presence of α-carboxyl groups of neuraminidase (EC 3.2.1.18)—susceptible *N*-acetylneuraminic acid (NANA, pK_a *ca.* 2.6; molecular weight 309) (Klenk and Lempfrid, 1957; Klenk and Uhlenbruck, 1958; Piper, 1957; Cook, Heard, and Seaman, 1961; Eylar *et al.*, 1962). Let it be assumed that the removal of 1 molecule of NANA (molecular weight 309) from the cell surface corresponds to the loss of 1 elementary electron charge as detected by cell electrophoresis. Therefore, in order to detect a 10% change in the electrophoretic mobility (or 10^7 electron charges) or, in other words, to determine 10^6 electron charges, only a minute amount of NANA need be removed from the surface:

$$\frac{309 \times 1.19 \times 10^6}{6.024 \times 10^{23}} = 6 \times 10^{-16} \text{ g.}$$

D. *The Scope of Cell Electrophoresis for Biological Systems*

Electrokinetic studies of some model compounds

The pH mobility plots over a wide range of pH values have yielded information about the electrokinetic properties of model chemical compounds of biological interest such as long-chain fatty acids, alcohols, and amines (Kruyt and Went, 1931; Kruyt and Van Gils, 1936; Growney, 1941; Bangham, Pethica, and Seaman, 1958; Ottewill and Wilkins, 1962;

Hollingshead, Johnson, and Pethica, 1965; Mehrishi, 1967; Mehrishi and Seaman, 1968). Such studies are useful in the interpretation of the behaviour of biological materials. Studies on quartz particles have also been carried out (Abramson, 1934; Sachtleben, 1965; Mehrishi and Seaman, 1966). Proteins can be studied by adsorbing them on quartz particles. The electrophoretic behaviour of γ-globulins adsorbed on quartz particles has been examined in connection with the studies on the adsorbed antibody on the cell surface (Forrester, Dumonde, and Ambrose, 1965; Sachtleben, 1965). The technique of cell electrophoresis has been used extensively to obtain information about the cell surface characteristics in general and to identify the cell surface groups of various pK values over the range 2–10. The literature is vast.

The following references may be consulted for the pH mobility relationships for (1) human erythrocytes (Heard and Seaman, 1960; Seaman and Heard, 1960), (2) human blood platelets (Kirschmann, Katchalsky, and de Vries, 1959; Seaman and Vassar, 1966), (3) human lymphocytes (Mehrishi and Thomson, 1968; Thomson and Mehrishi, 1969), and (4) human leucocytes (Vassar, Kendall, and Seaman, 1969). It must be pointed out, however, that reasonable criticisms about the interpretation of the electrokinetic data on cells subjected to extremes of pH values (such as below pH 4 or above 9) or harsh reagents such as the aldehydes (Heard and Seaman, 1961) could be made (Mehrishi, 1970a). It was emphasized that artefacts may be observed when cells such as lymphocytes are subjected to low pH values even for a short time (Mehrishi and Thomson, 1968; Thomson and Mehrishi, 1969). By electrophoretic examination of cells after treatment with suitable mild, specific reagents (experimental time 10–20 min), the chemical nature of the surface groups can be identified and their contribution to the electrokinetic charge calculated. The presence of positively charged amino groups (Mehrishi, 1970a) and sulphydryl groups (Mehrishi and Grassetti, 1969) on the surface of human blood platelets, lymphocytes, and Ehrlich ascites tumour cells has thus been established. Electrophoretic studies on cells after treatment with enzymes which will specifically remove cell surface groups has also yielded valuable information about the various anionogenic groups contributing to the electrokinetic charge as listed below:

(I) Neuraminidase-susceptible *N*-acetylneuraminic acid [NANA] α-carboxyl groups (p$K_a = 2.6$) on the surface of most mammalian cells examined:
  (i) human erythrocytes (Piper, 1957; Cook, Heard, and Seaman, 1961; Eylar *et al.*, 1962);
  (ii) human blood platelets (Madoff, Ebbe and Baldini, 1964; Seaman and Vassar, 1966);
  (iii) human lymphocytes (Mehrishi, 1968; Thomson and Mehrishi, 1969); and
  (iv) human leucocytes (Vassar, Kendall, and Seaman, 1969).

(II) Ribonuclease-susceptible phosphate groups on the surface of human lymphocytes and some tumour cells (Mayhew and Weiss, 1968).

(III) Alkaline-phosphatase-susceptible phosphate groups on the surface of human blood platelets (Mehrishi, 1970b).

However, caution is needed in the interpretation of the electrokinetic data on enzyme-treated cells because artefacts may be observed following the adsorption on the cell surface of the enzyme itself (Sachtleben, Gsell and Mehrishi, see Addendum) or the impurities therein, the autolysis products, intracellular products, or adsorption of material released by the action of the enzyme and conformational changes (Seaman and Uhlenbruck, 1963; see also Kraemer, 1968).

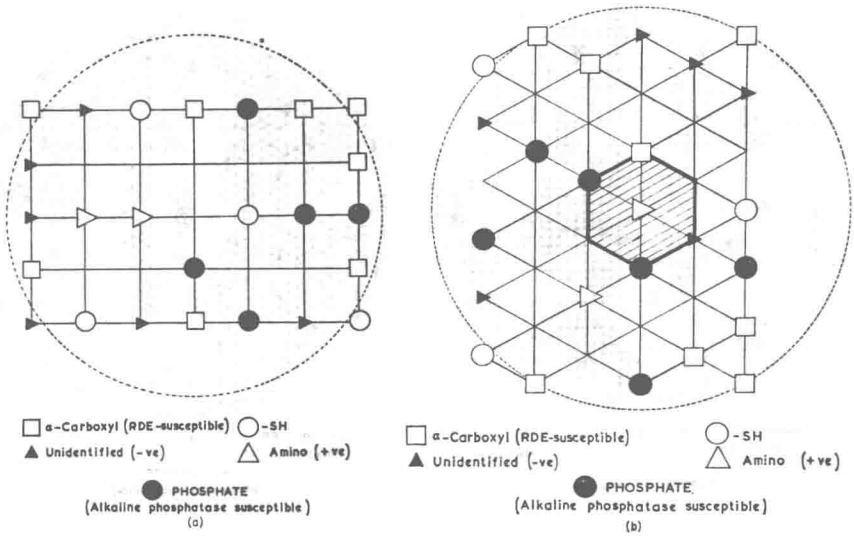

FIG. 2. Human platelet. The cell is assumed to have a spherical shape with a diameter of 28.27 μm^2. One symbol represents a cluster of 10^5 groups. (From Mehrishi, 1971b).

(a) Groups arranged at corners of squares.

(b) Groups arranged at corners of equilateral triangles (hexagonal packing).

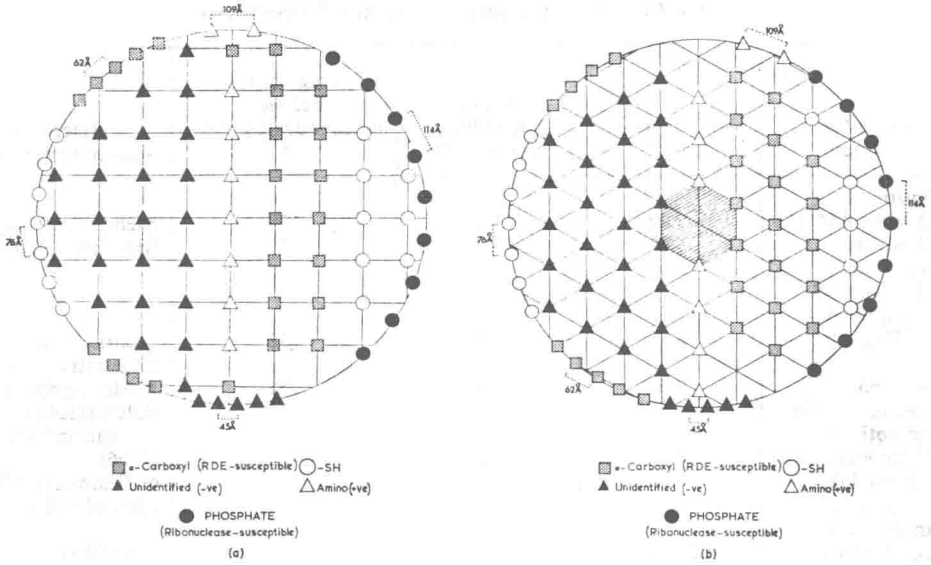

FIG. 3. Human lymphocyte. The cell is assumed to have a spherical shape with a diameter of 6 μm and a surface area of 113 μm^2. One symbol represents a cluster of 10^5 groups. (From Mehrishi, 1972a).

(a) Groups arranged at corners of squares.

(b) Groups arranged at corners of equilateral triangles (hexagonal packing).

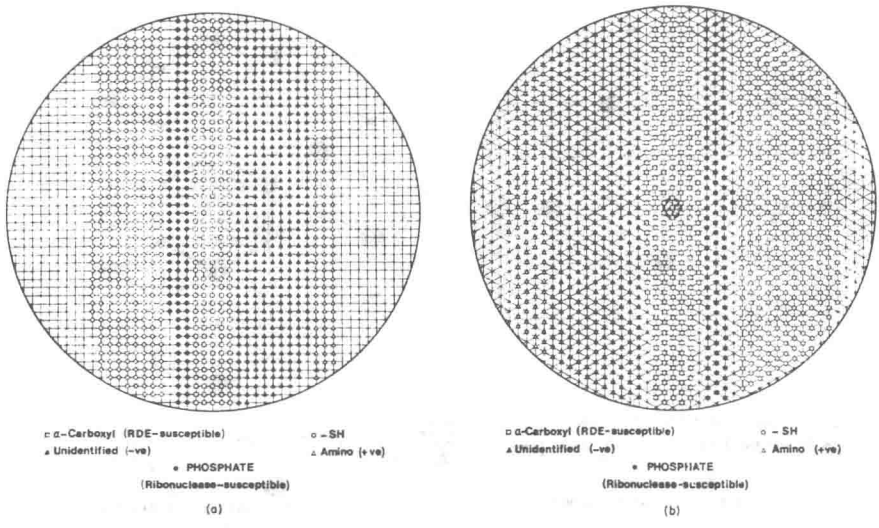

FIG. 4. Ehrlich ascites turnover cell. The cell is assumed to have a spherical shape with a diameter of 12 μm and a surface area of $\sim$ 900 μm^2 (range 600–1200 μm^2). One symbol represents a cluster of 10^5 groups. (From Mehrishi, 1972b).

(a) Groups arranged at corners of squares.

(b) Groups arranged at corners of equilateral triangles.

TABLE 1. CELL SURFACE MOLECULAR MOSAIC

Groups/Charges on the Surface of Human Thrombocytes[a]

Groups or charges	Number per cell ($\times 10^5$)	Area per group/charge ($Å^2 \times 10^3$)	Average distance between neighbouring groups (Å)	Reference (data processed from)
1. Positively charged AMINO	2.42	11.7	108	Mehrishi (1970a)
2. Phosphate (alkaline phosphatase-susceptible)	5.0[b]	5.7	75	Mehrishi (1970b)
3. —SH groups	2.75	10.3	32	Mehrishi and Grassetti (1969)
4. α-Carboxyl (neuraminidase-susceptible-*N*-acetylneuraminic acid)	9.5 8.9	2.98 3.2	55 56	Madoff, Ebbe, and Baldini (1964) Seaman and Vassar (1966) cf. Seaman (1967)
5. Unidentified anionogenic	6.53			Mehrishi (1970a)
6. Total electron charges	20.42			Mehrishi (1970a)
7. Net electron	18.0			Mehrishi (1970a) cf. Seaman (1967)

[a] Mean diameter, 3 μm (Wintrobe, 1967); surface area, 28.27 μm^2.

[b] Brossmer and Patscheke (1968) reported that the receptor sites on the thrombocyte surface can be removed by alkaline phosphatase and estimated to be 10^6–10^7 phosphate groups per thrombocyte.

TABLE 2. CELL SURFACE MOLECULAR MOSAIC
Groups/Charges on the Surface of Human Lymphocyte[a]

Groups or charges	Number per Cell ($\times 10^5$)	Area per group/charge (Å^2)	Average distance between neighbouring groups (Å)	Reference (data processed from)
1. Total electron charges	102.9			Mehrishi (1970a)
2. Electron charges contributing to the electrokinetic charge	93.4			Mehrishi (1970a)
3. α-Carboxyl (neuraminidase-susceptible-*N*-acetyl-neuraminic acid)	29.2	3880	62	Mehrishi and Thomson (1968) Thomson and Mehrishi (1969)
4. Phosphate (RNase-susceptible)	8.7	13000	114	Mayhew and Weiss (1968)
5. Positively charged amino	9.5	11900	109	Mehrishi (1970a)
6. Unidentified anionogenic	55.5	2050	45	Mehrishi (1970a)
7. —SH groups	19.8	5760	76	Mehrishi and Grassetti (1969)

[a] Mean diam., 6 μm; surface area, 113 μm^2.

TABLE 3. CELL SURFACE MOLECULAR MOSAIC
Groups/Charges on the Surface of Ehrlich Ascites Tumour Cells*

Groups or charges	Number per cell ($\times 10^7$)	Area per group/charge (Å^2)	Average distance between neighbouring groups (Å)	Reference (data processed from)
1. Total electron charges	8			Mehrishi (1970a)
2. Electron charges contributing to the electrokinetic charge	6.88			Mehrishi (1970a)
3. α-Carboxyl (neuraminidase-susceptible-*N*-acetyl-neuraminic acid)	2.35	3830	61.9	Cook, Heard, and Seaman (1962)
4. Phosphate (RNase-susceptible)	1.19	7550	86.9	Mayhew and Weiss (1968)
5. Positively charged amino	1.17	7700	87.6	Mehrishi (1970a)
6. Unidentified anionogenic	3.91	2300	48	Mehrishi (1970a)
7. —SH groups	3.69	2440	49.4	Mehrishi and Grassetti (1969)

* Camb. Univ. Dept. of Radiotherapeutics strain of tumour.

The number of the various types of groups (at least five: pK values 2.6; *ca.* 4, and above 9) on a per cell basis and their relative contributions to the electrokinetic make-up have been calculated and two possible arrangements for their distribution in clusters in the cell periphery have been proposed for (i) human blood platelets (Mehrishi, 1971a,b) (Table 1; Fig. 2), (ii) human lymphocytes (Mehrishi, 1972a) (Table 2; Fig. 3), and (iii) Ehrlich ascites tumour cells (Mehrishi, 1972b) (Table 3; Fig. 4). In parallel studies on human erythrocytes, amino, —SH, or phosphate groups have not been detected within 5–10% of 10^7 which is the limit of reliable detection by cell electrophoretic method. In other words, if the numbers of these groups is below, say, 5×10^5 to 10^6 groups, they will not be detected by cell electrophoresis and their contribution will not be significant (also see Fig. 16, p 28).

III. THE APPARATUS AND THE EXPERIMENTAL TECHNIQUE

A. *The Microscope Method of Cell Electrophoresis*

For the determination of electrophoretic mobilities from which zeta potentials (mV), surface charge densities (e.s.u./cm^2), and electron charges are evaluated, several types of apparatus have been designed. Basically the cell electrophoresis apparatus consists of a transparent chamber equipped with reversible electrodes at the two ends of the chamber mounted on the stage of a microscope. When the chamber is filled with a suspension of particles or cells, the migration of the cells under the influence of an applied electric field can be observed by means of a microscope under controlled conditions. A calibrated graticule is mounted in the ocular of the microscope so that the rate of migration (μm/sec) can be determined with a stop-watch. From the knowledge of the field strength (V/cm), the electrophoretic mobility (μm/sec/V/cm) can be calculated. Several types of electrophoresis chambers have been used. Recent publications describe the essentials of two types of apparatus equipped with an electrophoresis chamber of a circular cross-section (termed a cylindrical cell) (Bangham *et al.*, 1958; review: Seaman, 1965; Meherishi, 1971c) and a rectangular cross-section (Sachtleben *et al.*, 1961, 1967; Fuhrmann and Ruhenstroth-Bauer, 1965).

Experiments may be carried out at constant voltage with the cylindrical cell. With a rectangular cell the measurements are made at constant current, but it is essential to determine the specific resistance of the suspending medium. Thus an extra measurement of a temperature-dependent parameter, the specific resistance, is involved. Seaman (1965) has discussed the advantages and disadvantages of the two types of chambers. I should recommend the use of a cylindrical cell (Figs. 5, 6).

For the theoretical treatment of the subject and other details see Brinton and Lauffer (1959) and Shaw (1969).

B. *Comments on the Experimental Procedure*

On several grounds it was decided to use the cylindrical cell with silver–silver chloride–potassium chloride (saturated) electrodes (Seaman and Heard, 1961). However, a number of shortcomings of the electrophoresis cell was encountered. Its performance was not trouble-free. Valuable biological material, sometimes from patients in a particular clinical state,

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