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Biochem. J. (1962) **84**, 513

The Surface Properties of some Neoplastic Cells

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It was suggested by Bangham & Pethica (1960), as a result of electrophoresis experiments with mouse Ehrlich ascites-tumour cells, that the surface-charged groups on the tumour cells were phosphatide in character, and almost indistinguishable in this respect from the surface charges of the lymphocyte, which circulates freely in the blood (Bangham, Pethica & Seaman, 1958*b*). The phosphatide surface groups may account also for the non-adhesiveness of the tumour-cell membrane; studies of the surface properties of tumour cells are thus of interest in investigations of metastasis and malignancy.

The object of the following study has been to extend our basic knowledge of the tumour-cell surface. Investigations have been made of the effect of pH, ionic strength, and of anionic and cationic adsorption on the electrophoretic mobility of mouse Ehrlich ascites-tumour cells. Similar, though less extensive, experiments have been carried out with Klein lymphosarcoma and sarcoma 37.

EXPERIMENTAL

Preparation of cell suspensions. The neoplastic cells, mouse erythrocytes and mouse-liver cells were washed three times with 100 vol. of 0·145M-NaCl; after each washing the cells were centrifuged at 1500*g* for 3 min.

Ehrlich ascites-tumour cells. Two sources of Ehrlich ascites-tumour cells were used. Most of the results were obtained with tumour cells from C3H mice provided by Dr D. Thomason, Christie Hospital and Holt Radium Institute, Manchester (Thomason & Schofield, 1961). Cells removed from white mice, provided by Mrs Simon-Reuss, Department of Radiotherapeutics, Cambridge, gave a second source. The nature of the source is indicated in this paper by (*a*) and (*b*) respectively.

Preliminary experiments indicated that there was no variation in the mobility of the cells washed after they had been left suspended in the ascitic fluid at room temperature for 4 hr. After this period 3% of the cells were non-viable, as shown by the Nigrosine technique (Kaltenbach, Kaltenbach & Lyons, 1958). Hence the cells were left in the ascitic fluid for no longer than 4 hr. and a cell suspension was prepared for an experiment, as above, just before use.

Klein lymphosarcoma and sarcoma 37. These were obtained from Dr E. J. Ambrose, Chester Beatty Research Institute, London.

Stearic acid dispersions. These were prepared by shaking stearic acid with warm water in a stoppered all-glass tube.

Materials. All solutions were prepared in water distilled from alkaline $KMnO_4$ and redistilled from a Pyrex still. Ethidium bromide and Prothidium bromide (Watkins & Woolfe, 1956) were supplied by Boots Pure Drug Co. Ltd.; Antrycide dimethyl sulphate (Curd & Davey, 1949; Ainley, Curd, Hepworth, Murray & Vasey, 1953), Paludrine hydro-

chloride (Curd & Rose, 1946*a*, *b*), 4-methylPaludrine (Curd, Hendry, Kenny, Murray & Rose, 1948) and Paludrine metabolite (Carrington, Crowther & Stacey, 1954) were obtained from the Pharmaceuticals Division, Imperial Chemical Industries Ltd., as were the Antrycide analogues, referred to in this paper as (A) and (B) respectively, i.e. 1-allyl-4-allylamino-6-(2-amino-1,6-dimethylpyrimid-4-ylamino)quinaldine 1,1′-di-iodide and 4-amino-6-(6-methyl-2-methylthiopyrimid-4-ylamino)quinaldine 1,1′-dimethiodide (W. G. M. Jones, personal communication).

Electrophoretic mobilities. Measurements were made at 25° in the apparatus described by Bangham, Flemans, Heard & Seaman (1958*a*). The mobilities, $\bar{V}$, of the cells were calculated in μ/sec./v/cm. and converted into ζ-potentials by use of the Helmholtz–Smoluchowski equation: $\zeta = (4\pi\eta/D)\,\bar{V}$, where η and D are the viscosity and dielectric constant of the suspending medium. Each mobility value was calculated by timing the movements of 14–20 cells. The charge density was calculated from the simplified Gouy equation: $\sigma = 3{\cdot}53 \times 10^4 \times I^{\frac{1}{2}} \times \sinh(\zeta/51{\cdot}3)$, where ζ is the electrokinetic potential in millivolts, I the ionic strength and σ the charge density in e.s.u./cm.^{2}.

Prothidium bromide adsorption. Washed Ehrlich ascites cells (*a*) were suspended in solutions of Prothidium bromide of known concentrations for 15 min. (this time-interval was chosen as being comparable with the time required for an electrophoresis experiment). The suspension was then centrifuged and the concentration of the Prothidium bromide remaining in the supernatant layer determined spectrophotometrically with a Hilger Spekker instrument.

RESULTS

Fig. 1 is a histogram demonstrating the variation in mobility of Ehrlich ascites-tumour cells (*a*) in iso-osmotic sodium chloride solution during a 12-month period in which the experiments were performed. The pH–mobility curves for the cells as a function of I are shown in Fig. 2. Similar curves for the Klein lymphosarcoma and sarcoma 37 are given in Fig. 3. Fig. 4 shows the variation in mobility of Ehrlich ascites-tumour cells (*a*) with

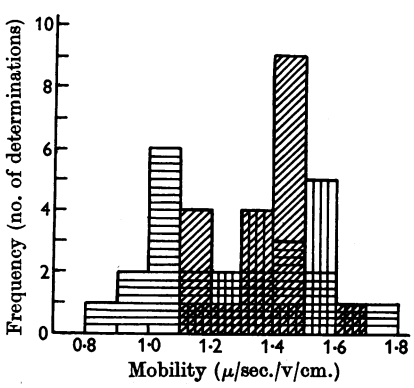

Fig. 1. Variation in the mobility of Ehrlich ascites-tumour cells (*a*) during three consecutive periods of 4 months each: ⊟, first; ▨, second; ▥, third.

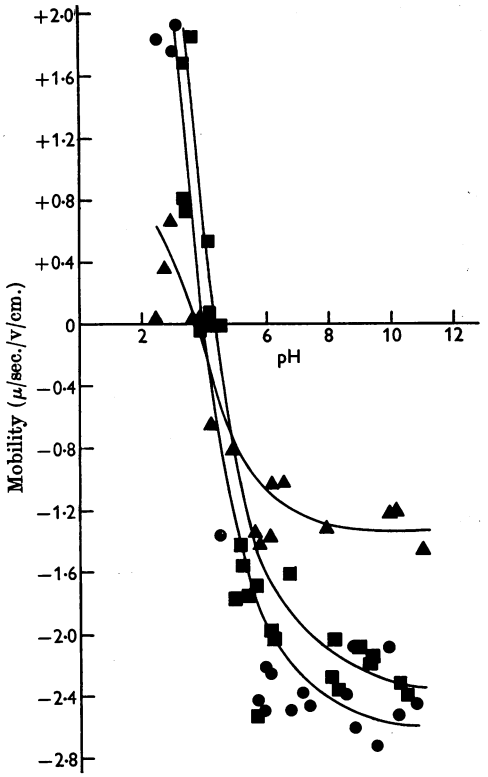

Fig. 2. Effect of pH on the mobility of Ehrlich ascites-tumour cells (*a*) as a function of I: ▲, 0·145; ■, 0·0145; ●, 0·003625. Solutions were made up with HCl, NaCl and NaOH as required, unbuffered.

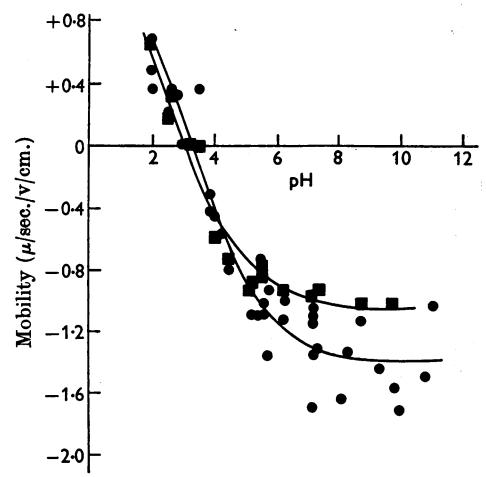

Fig. 3. Effect of pH on the mobility of Klein lymphosarcoma (■) and sarcoma 37 (●). The solution (unbuffered) had I 0·145.

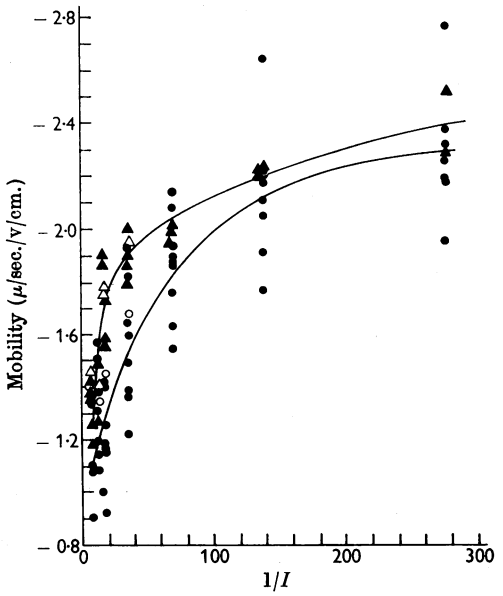

Fig. 4. Variation in the mobility of Ehrlich ascites-tumour cells (*a*) with $1/I$. ● and ○, NaCl; ▲ and △, NaI. ● and ▲, obtained with cells from many different mice; ○ and △, obtained with cells from one mouse.

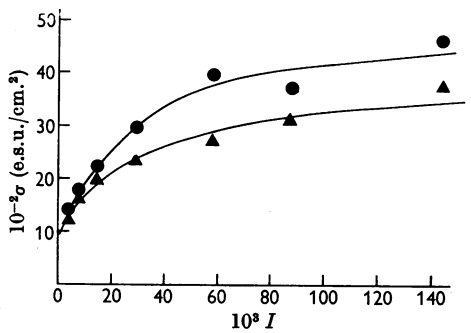

Fig. 5. Adsorption isotherms showing the variation in surface-charge density, σ, with ionic strength, I. ●, Cl^-; ▲, I^-. The points represent the values of σ calculated from the mean mobility values shown in Fig. 4.

$1/I$ for sodium iodide and sodium chloride. The values of the mobility corresponding to these curves have been used in the estimation of the surface charge to give the nominal adsorption isotherms of Fig. 5.

No significant difference was found between mobilities obtained from experiments where the iso-osmoticity was maintained constant with sucrose or where a buffered medium was used (0·3 mM-sodium hydrogen carbonate), as distinct from experiments carried out in non-iso-osmotic or unbuffered conditions.

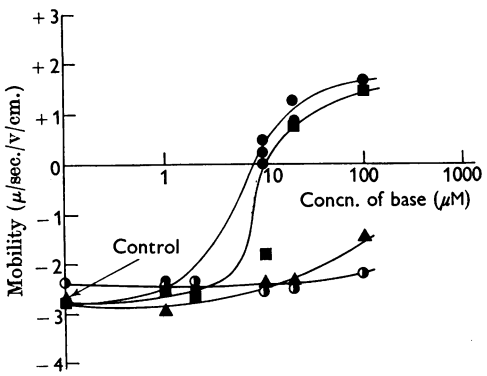

Fig. 6. Variation in mobility of stearic acid particles with concentration of base. ●, Prothidium; ■, Antrycide; ◑, Antrycide analogue (A); ▲, Antrycide analogue (B). The control was obtained with 0·145 M-NaCl.

Fig. 6 gives the variation in mobility of a stearic acid dispersion with concentration of some organic bases, at pH 7·2. Table 1 shows the percentage reduction in mobility of the various tumour cells on the addition of certain organic bases at pH 7·0. In some experiments the cells were rewashed with sodium chloride solution after the addition of the organic base and the mobility was redetermined. Mouse serum (1:100 dilution) was added in other experiments. Where investigated, the corresponding reductions in mobility of the mouse erythrocyte and liver cell is quoted.

In Table 1 the authors correct the report by Bangham & Pethica (1960) that mM-quinine gives a charge reversal of the tumour cell.

DISCUSSION

That caution has to be exercised in interpreting the electrophoretic data of the tumour cells in quantitative terms is obvious from the wide range of mobilities of Ehrlich ascites cells in sodium chloride solution (Fig. 1). The trend of the results obtained from repeated experiments with cells from different mice gives a better guide for a qualitative interpretation.

The Ehrlich ascites-tumour cells exhibit an isoelectric point in the pH region 3·5–4·5, with no observable dependence on I over the range studied (Fig. 2). An isoelectric point of pH 3·0–3·5 is found for the Klein lymphosarcoma and sarcoma 37 (Fig. 3). The pH–mobility curves for lecithins show the same general shape as for the tumour cells, and have isoelectric points of about pH 2·5–3·5 (Bangham *et al.* 1958*b*). Values of the isoelectric point as high as pH 4·0–4·5 for lecithins are given by Seaman (1958).

Table 1. *Percentage reduction in the negative mobility of cells with different organic bases*

Full experimental details are given in the text. The organic bases were used in 0·145 M-NaCl solution at pH 7·0. The numbers of experiments are given in parentheses. E.A.C., Ehrlich ascites cells; R.B.C., mouse erythrocytes; L.C., mouse-liver cells; S37, sarcoma 37 cells; K.L.S., Klein lymphosarcoma cells. +, Increase in negative mobility.

Base	E.A.C. (*a*)	E.A.C. (*a*) rewashed	R.B.C. (*a*)	E.A.C. (*b*)	R.B.C. (*b*)	L.C. (*b*)	S37	S37 rewashed	S37 +serum	K.L.S.	K.L.S. +serum
p-Toluidine hydrochloride (mM)	8·5 (1)	—	—	—	—	—	—	—	—	—	—
Pyridine (mM)	8 (1)	—	—	—	—	—	—	—	—	—	—
Quinine dihydrochloride (mM)	33 (2)	—	—	11 (1)	—	—	—	—	—	—	—
Paludrine hydrochloride (0·1 mM)	2 (4)	—	3 (1)	—	—	—	19·5 (1)	—	—	—	—
Paludrine metabolite hydrochloride (0·1 mM)	1 (2)	—	—	—	—	—	—	—	—	—	—
4-Methyl]Paludrine hydrochloride (mM)	0 (3)	—	+3·5 (1)	—	—	—	—	—	—	—	—
Antrycide dimethyl sulphate (0·1 mM)	28 (3)	—	+0·5 (1)	21 (2)	8·5 (1)	+2 (1)	42 (2)	+3 (1)	+40·5 (1)	12 (1)	2·5 (1)
Antrycide analogue (A) (0·1 mM)	2·5 (4)	—	+2·5 (2)	+11·5 (1)	0 (1)	+12 (1)	—	—	—	—	—
Antrycide analogue (B) (0·1 mM)	—	—	—	5 (1)	1·5 (1)	+12·5 (1)	22·5 (3)	—	0 (1)	3·5 (1)	12·5 (1)
Ethidium bromide (0·1 mM)	+9 (2)	—	—	—	—	—	—	—	—	—	—
Prothidium bromide (0·1 mM)	48 (7)	29 (3)	+1 (1)	31 (3)	8 (1)	+6·5 (1)	46 (3)	5 (1)	36 (1)	3 (2)	4·5 (1)
Dodecyltrimethylammonium bromide (0·1 mM)	+2 (2)	—	—	—	—	—	—	—	—	—	—

Previous workers have discussed in detail the interpretation of simple adsorption isotherms on biological surfaces (Douglas & Shaw, 1957; Haydon, 1961). Accepting the limitations of this method, the only interpretation we wish to place on the data from our experiments (Fig. 5) is to suggest that the cell surface of the Ehrlich ascites-tumour cells shows an electrophoretic specificity towards anions, since the nominal adsorption isotherms of I^- and Cl^- ions are different. This result for the Ehrlich tumour cells is in contrast with the findings of Heard & Seaman (1960) for erythrocytes.

The differences in effect of the various organic bases on the surface of the tumour cells are very marked, the bases showing a variation in their specificity, not only towards the cells of one neoplasm, but also towards cells of different neoplasia. In the Ehrlich ascites tumour and sarcoma 37, Antrycide and Prothidium are the most effective in reducing the negative charge on the tumour-cell surface at concentrations similar to those used in trypanosome therapy. The Antrycide analogues (inactive in the treatment of trypanosomiasis), bases of the Paludrine family and the more simple organic bases have little effect on the mobility of the cells. Compared with the Ehrlich ascites tumour and sarcoma 37, the Klein lymphosarcoma shows little response towards any of the organic bases. The simultaneous injection of Antrycide or Prothidium with Ehrlich ascites-tumour cells into white mice did not increase the survival time of the mice.

The bases are easily removed from the cell surface of sarcoma 37 by rewashing with iso-osmotic sodium chloride solution, but only partially from the surface of the Ehrlich tumour cells. Their effectiveness in altering the mobility of a cell is not enhanced in the presence of serum.

Marked changes in the mobility of erythrocytes and liver cells in the presence of the organic bases are due to increases in the negative charge on the surface of the cells.

The difference in effect of Antrycide, Prothidium and the Antrycide analogues on the electrophoresis properties of a model system is illustrated by the use of a stearic acid dispersion (Fig. 6). Both Antrycide and Prothidium cause a charge reversal at a concentration of 10 μM, whereas the analogues have a negligible effect.

The results of our experiments suggest that the organic bases show adsorption specificity on different cell membranes. That the effect of these bases will not only depend on the adsorption on the external surface of the membranes is shown by spectroscopic experiments to measure the whole uptake of Prothidium by Ehrlich ascites cells (*a*). At an extracellular equilibrium concentration

of 10 mM-Prothidium, the uptake of the base in a washed cell suspension was equivalent to 1 molecule/30 Å^2 of external surface. This result is to be compared with the calculated uptake of the same base at a concentration of 10 mM as shown by electrophoresis, i.e. 1 molecule/3000 Å^2.

SUMMARY

1. The effect of pH, ionic strength, and of anionic and cationic adsorption on various neoplastic cells has been examined electrophoretically.

2. Isoelectric points in the pH region 3·0–4·5 were found for the Ehrlich ascites-tumour cell, Klein lymphosarcoma and sarcoma 37.

3. The cell surface of the Ehrlich ascites-tumour cell appears to adsorb I^- ion more than Cl^- ion.

4. Marked differences in the effect of various organic bases on the mobility of the tumour cells were observed. In particular, Antrycide and Prothidium at a concentration of 10 mM give a reduction of 30–50 % in the mobility of the cells of the Ehrlich ascites tumour and sarcoma 37, whereas the analogues (which are biologically inactive) have a negligible effect.

5. The organic bases show adsorption specificity on different cell membranes.

6. The total uptake of Prothidium by Ehrlich ascites cells is much greater than the amount adsorbed on the external membrane.

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Some Properties of Glyoxylate Reductase in Cell Extracts of *Pseudomonas* sp.

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During the investigation of the operation of the glyoxylate cycle in a species of soil *Pseudomonas* (Hullin & Hassall, 1960, 1962), it became apparent that glyoxylate was utilized by cell extracts in reactions other than for the synthesis of malate. The enzyme responsible reduces glyoxylate to glycollate, and hence, by analogy with the plant enzyme, has been named glyoxylate reductase (glycollate–NAD oxidoreductase, EC 1.1.1.26); a description of some of its properties is given in this paper. The action of this enzyme, in conjunction with isocitrate lyase, will adequately explain the occurrence of glycollate in bacterial systems when acetate is the source of carbon for growth (Ajl, 1952; Bolcato, de Bernard & Leggiero, 1957

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