Electrophoretic Mobility of Irradiated Erythrocytes and Ehrlich Ascites Tumour Cells

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ABSTRACT

Changes in the electrophoretic mobility of mammalian cells following irradiation with X, γ and β rays are reported. The cells investigated were human and cockerel erythrocytes and Ehrlich Ascites Tumour Cells. The electrophoretic mobility of the cells showed a biphasic response with dose. The general trend of the response was that the cockerel erythrocytes showed an initial increase in mobility, then a return to normal levels. Human erythrocytes and ascites cells exhibited an initial decrease in mobility before returning to the control values. The X and γ dose levels required for these changes were similar and substantially greater than when β radiations were used.

1. INTRODUCTION

Studies of radiation-induced changes in the electrophoretic mobility of suspended colloidal particles and various animal cells have produced diverse results both with regard to the dose required to elicit measurable mobility change and to the possible cyclical nature of the response. Crowther, Liebman and Lane (1937), examining graphite particles, recorded cyclical mobility changes with increasing radiation doses. Similar cyclical changes are reported for Ehrlich ascites tumour cells (Stein, Seaman and Heard, 1962). The ascites cell mobilities increased to a maximum at 600 rads, then decreased

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to values similar to those of control cells at 900 rads; further dose increases produced secondary maxima and minima at 1200 rads and 1500 rads respectively. However, Stein, Seaman, Mehrishi and Simon-Reuss (1966) failed to confirm the results and suggested that an infection of the original cell line had caused the anomalous results. They reported that doses in excess of 2000 rads were required to produce a mobility decrease in ascites cells whilst the mobility of human erythrocytes remained unaffected by doses of up to 20 K rads. In contrast Tribukait (1968) reported cyclical mobility changes in human erythrocytes in response to X-irradiation doses of less than 100 rads.

The present investigation aims to clarify these conflicts by examining the effects of various radiation types (X, γ and β-rays) on three cell species: (a) Human erythrocytes, (b) Nucleated cockerel erythrocytes, and (c) Ehrlich murine ascites tumour cells.

2. MATERIALS AND METHODS

Human and cockerel erythrocytes were collected by venipuncture, then defibrinated with glass beads in a glass phial. The samples were centrifuged and the cells washed three times, then re-suspended in a phosphate buffer similar to that used by Bangham (1958): (M/15 KH_2PO_4 and M/15 Na_2HPO_4 in volumetric proportion 1:4). Ehrlich ascites cells were cultured in I.C.I. albino mice. 0.1 ml cell suspension was injected under light anaesthesia into the peritoneal cavity (day 0) and the ascitic fluid extracted asceptically from mice killed by ether asphyxiation (day 8).

The radiation sources used were as follows:

(a) X-irradiation generated at 250 keV, 15 mA (H.V.T. 0.36 mm Cu) with a dose rate of 600 rads min^{-1};

(b) γ-irradiation from a 1000 Ci, ^{60}Co source delivering a dose rate of 5150 rads min^{-1};

(c) β-irradiation from a ^{32}P source. Irradiation was carried out by suspending cells in a known volume of ^{32}P at a specific activity

of 1 mCi ml^{-1}. Assuming uniform mixing of the phosphorus, the calculated dose rate was 20.4 rads min^{-1}.

The X and γ irradiations were carried out in standard glass phials. Whilst both erythrocytes species were suspended in the phosphate buffer during irradiation, the ascites cells were irradiated in the ascitic fluid. Several irradiations were repeated under well oxygenated conditions by passing pure oxygen through the suspension. After irradiation all cell species were washed three times in phosphate buffer.

The determinations of electrophoretic mobility were made at constant temperature (37.0°C) in a rectangular electrophoretic cell[1] with a potential gradient of 4.3 V cm^{-1}. The passage of an individual cell was timed over a total distance 100 μ, the potential gradient being reversed after 50 μ. At least twenty replicate measurements were made on each sample; part of each sample was retained for later determination of the non-irradiated control mobilities.

To obtain absolute values of electrophoretic mobility the measurements were made at the stationary level where the electrosmotic and return flows equilibrate. For a cylindical tube, internal radius 'R', Bangham, Heard, Seaman and Flemans (1958), recorded the stationary level at a distance 0.707 R from the axis of the tube. For a rectangular cell of the type used in the present study the stationary level may be accurately determined by empirical means. Cell mobilities are determined at two different pHs and plotted against the square of displacement from the longitudinal axis of the tube. This gives two straight lines of different slope where the point of intersection corresponds to the stationary level. Figure 1 illustrates the regression line of human erythrocyte mobilities for varying focal planes in the rectangular cell at pH 7.35 and pH 6.60. The regression coefficients are -0.089 and -0.044 respectively, their intersection at the stationary level gives a mobility, m = -1.298 u $s^{-1}v^{-1}$ cm which is similar

[1] Rank Bros., Mk.II, manufactured by Rank Bros., Cambridge, U.K.

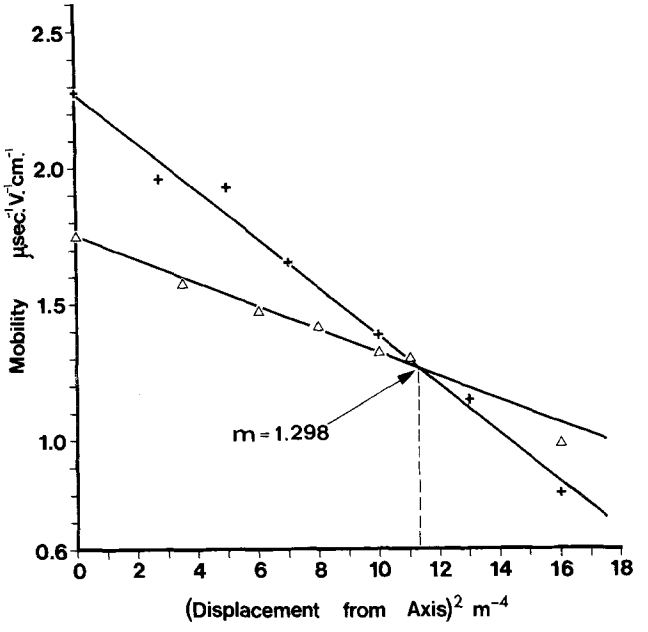


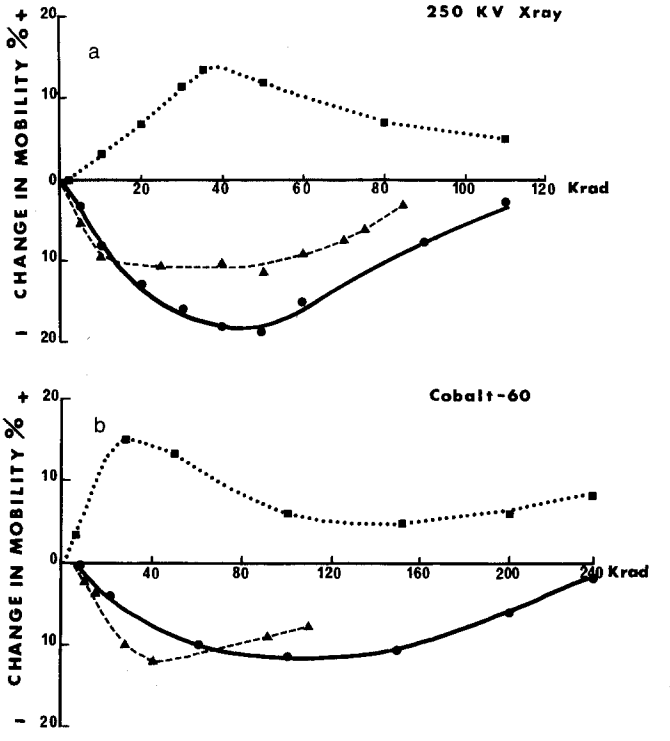
Fig. 1 Human erythrocyte mobilities at pH 7.35 (+) and pH 6.60 (△) at different focal planes in the rectangular electrophoretic mobility cell. The absolute mobility, m at the stationary level is the value at the intersect and in this case is -1.298 $\mu\ s^{-1}v^{-1}$ cm.

to previously reported values (Loveday and James, 1957; Bangham and Heard, 1958).

Mobility determinations on ascites cells were confined to within three hours of collection from the host since cells exhibit diminished viability after intervals in excess of four hours (Bangham, Glover, Hollingshead and Pethica, 1962). For each series of experiments the 'viability' of random ascites cell samples was tested with Tryphan Blue which indicates gross changes in membrance permeability.

3. RESULTS

The mean mobilities for non-irradiated(control) cells were as follows: human erythrocytes -1.30 ± 0.03 $\mu\ s^{-1}v^{-1}$ cm, cockerel erythrocytes -1.28 ±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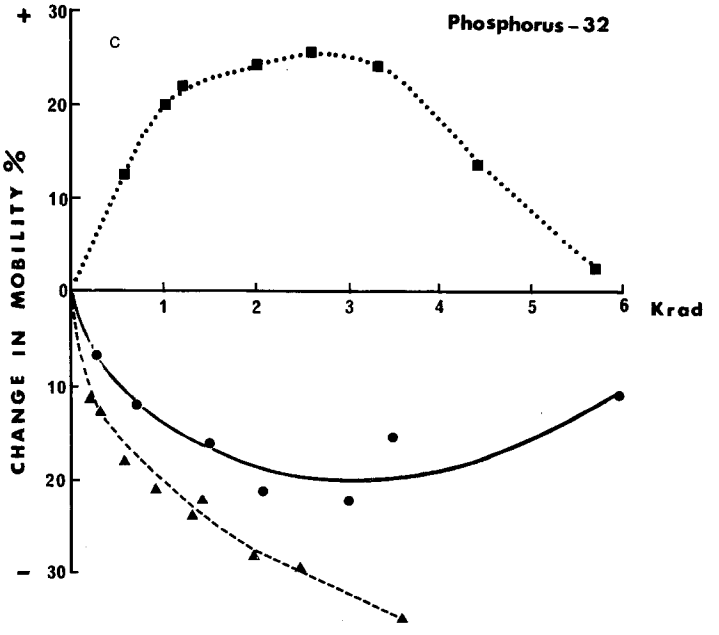
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0.03 $\mu s^{-1}v^{-1}$ cm and Ehrlich murine ascites tumour cells -1.90 $\pm$ 0.05 $\mu s^{-1}v^{-1}$ cm. The negative sign indicates a nett negative cell surface charge.

Figures 2a, b and c illustrate the effects of X, γ and β irradiation respectively, on the three cell species. The magnitude of the mobility changes was unaffected by oxygenation during irradiation. With the exception of β irradiated ascites cells, the mobility curves for all the cells indicate a biphasic response to the radiation although there are some differences between the various radiations in terms of the dosages required to elicit a given change. This is particularly true for the case of ^{32}P compared with the X or radiations. The maximum observed mobility change was 30% for ^{32}P and 10-20% for X and γ irradiations.

X and γ irradiations produced essentially the same response in all the cells, the maximum mobility change taking place at 40-60 K rads, followed by a return towards the values of the irradiated control cells. The obvious difference between the various cells was that the mobility of cockerel erythrocytes was increased by irradiation whilst the mobility of human erythrocytes and ascites cells decreased. This response is somewhat similar to that following treatment with neurominidase which hydrolyses glycoproteins in the cell wall (Seaman and Uhlenbruck, 1963). Despite this obvious difference between human and cockerel erythrocytes, there is some degree of similarity between these two cells in that the response curves are similar in shape, ignoring the sign, for all three types of radiation. The response curves of the ascites cells were somewhat similar to those of human erythrocytes following X and γ irradiation but β irradiation resulted in a continuing decrease in mobility over the dose range investigated (0 - 4 K rad). The dose required to produce a specific

Fig. 2 Mobility changes of human (●) and cockerel (■) erythrocytes and Ehrlich ascites cells (▲) following irradiation with (a) X-rays, (b) ^{60}Co γ rays and (c) β-rays from ^{32}P.

mobility change was less than that for either X or γ irradiation by a factor of 20. The β irradiation was carried out under markedly different conditions to the X and γ ray treatments and it is probably that ^{32}P is actively incorporated into the cell and therefore its effects may not be directly compared to those of the X and γ irradiation.

4. DISCUSSION

The values for the electrophoretic mobility of non-irradiated human erythrocytes are similar to those of other workers (Loveday and James, 1957; Bangham and Heard, 1958). The control mobilities of cockerel erythrocytes and of Ehrlich ascites cells exceeded previously reported values (Seaman and Uhlenbruck, 1963; Taylor and Newman, 1972). The reason for this is unknown, although Bangham et al (1962) recorded marked variations in tumour cell mobilities over a period of months. In addition the mobility measurements were made at $37^{o}C$ in contrast to the more generally adopted value of $25^{o}C$. However, the present results remain valid as mobility changes are expressed as percentage change relative to the control mobility.

The mobility changes reported here are broadly similar for all three types of radiations although lower β doses were required than with X or γ radiations. Human erythrocytes and ascites cells showed a similar response to increasing dose, namely a decrease in mobility by 10 - 20% and then a gradual return towards control values. A decrease in the mobility of irradiated ascites cells has been previously reported (Stein et al, 1966) but there was no evidence in this study of cyclical changes, i.e. a periodic increase and decrease in mobility as observed by other workers (Crowther et al, 1937; Stein et al, 1962; Tribukait, 1968).

An explanation of these changes requires a more detailed knowledge of the chemical structure of the cell surface than is available at the present time. Seaman and Heard (1960) postulated that the cell surface comprises a molecular matrix with charged systems located in the near-surface membrane regions and probably contributing to the electrokinetic properties at the

plan of shear. The negative mobility of cells is attributed to the presence of acidic ionogenic groupings on the surface. The major contribution to the nett negative charge is from the carboxyl groups of N-acetylated sialic acid although carboxyl groups of some amino acids, phosphate and amine groups make some contribution.

The nett surface charge generated by surface ionisation in aqueous solutions, attracts cations forming a surrounding ionic atmosphere, the double layer. Movements of a cell under an applied electric potential is accompanied by movement of part of the double layer; the plane between the 'captured' fluid and the fluid of the bulk phase is termed the hydrodynamic slip plane. Electrophoretic mobility studies provide data on potentials (zeta potentials) at the slip plane periphery. Several assumptions are required to estimate the surface potential. Electrophoretic measurements made under various conditions of ionic strength, pH value and enzymatic treatment suggest that the charged groups of the cell surface are not located in a single plane, rather at different levels over 1 - 2 nm depth. Cell electrophoretic mobility depends on the number of ionogenic groups contributing to the charge at the slip plane. Mobility changes may be produced by absorption of ions, changes in the effective thickness of the electrical double layer, deletion of components from the membrance or by rearrangement of the molecular architecture of the surface. This range of causal factors which can elicit a mobility change makes it difficult to define the sequence of events produced by radiation treatment. It should be noted however that Yamada and Yamada (1973) found biphasic mobility responses similar to those described here in malignant and normal rate cells treated with plant agglutinins such as concanavalin (Con. A.). They suggested that low concentrations of Con. A. bind to specific sites on the cell surface and change the distribution of glycoproteins. Sialic acid groups then shift reactively into the electrokinetic plan of shear, so resulting in a mobility increase of up to 20% of the control value, which is similar to changes

found in this present study. At high concentrations the Con. A. molecules cover negative elementary charges at the cell surface resulting in a mobility decrease. These changes are similar to those found for irradiated cockerel erythrocytes. The opposite response of human erythrocytes and ascites cells following radiation and neurominidase treatment (Seaman and Uhlenbruck, 1963) indicates a fundamental difference in surface structure between mammalian and avian cells.

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