Anionic Polymers

IV. Microelectrophoresis of Ascites Tumor Cells and the Effect of Polyxenylphosphate*

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The distribution of radioactivity in tissues, particularly in tumors, of mice given injections of certain preparations of the anionic polymer, polyxenyl phosphate (PXP)* labeled with P32, are explained neither on the basis of reticuloendothelial activity nor solely by hydrolysis and subsequent uptake of P32-orthophosphate (12). One of several explanations might be that PXP is bound to cell surfaces. To explore this possibility, Ehrlich and Sarcoma 180 ascites tumors were selected so that independent neoplastic cells could be used to determine the extent of surface binding by means of microelectrophoresis. Microelectrophoresis has been extensively used to study the surface characteristics of cell structures (2-4, 7, 11, 13-15). The net charge of the surface of a microscopically visible cell may be calculated from its rate of migration in an electric field (1), provided the ionic strength and tonicity are known. If PXP is bound to the cell surfaces, the mobility of the ascites cells toward the anode would be increased. Thus, the quantity of polymer bound to the cell surfaces may be estimated from the dimensions of the tumor cells and the charge density of the polymer.

MATERIALS AND METHODS

Tumor cells.—The source and propagation of the mouse tumors have been described (12). Ascites tumor cells were collected by flushing of the peritoneal cavity with 0.9 per cent saline and centrifugation of the diluted ascitic fluid. All preparations of these cells were washed 3 times with about 8 volumes of 0.9 per cent saline. A 1:10 suspension of the cells in 0.9 per cent saline or 5.4 per cent glucose was gently agitated and then slowly forced through a 27-gauge needle to disperse cell clumps. The resultant suspension was then diluted with appropriate buffers.

Buffers.—For studying the effect of ionic strength on mobility, various dilutions (7) of stock phosphate-saline buffer2 with 5.4 per cent glucose were prepared. To 60 ml. of these glucose-buffer mixtures was added 1 ml. of a 1:10 dilution of ascites cells in 5.4 per cent glucose. To study the effect of pH on the mobility of ascites cells, a series of buffers of essentially constant ionic strength and tonicity was prepared. For pH values from 5.6 to 8.0, 10 ml. of suitable mixtures of M/15 KH2PO4 and M/15 NaHPO4 were diluted to 100 ml. with 1 per cent NaCl. For pH values from 8.0 to 8.5, 20 ml. of suitable mixtures of 0.2 m sodium acetate and 0.2 m acetic acid were diluted to 100 ml. with 0.06 per cent NaCl. For use, these solutions were diluted with 3 volumes of 5.4 per cent glucose solution, giving isotonic solutions of approximately 0.04 ionic strength.

PXP solutions.—The PXP preparation employed throughout this study had an equivalent weight of 240, and contained 22 per cent of the total acidity (up to pH 8.5) as phosphomonoester groups and contained 12 per cent by weight of terminal p-biphenolic groups. The preparation of PXP-B fraction is described in the preceding paper (14). Cell suspensions were incubated at room temperature with 2 volumes of buffer plus PXP or other additions for 4 hours, then diluted with 20 volumes of glucose-buffer solution and the electrophoretic mobilities measured. The specific conductance of the suspension was unaffected by the concentrations of PXP used. Both the uptake of P32 as measured by disappearance of radioactivity from the supernatant and the increased mobility caused by PXP were determined with the same suspension of S180 cells.

Cell population.—Cell suspensions were diluted with 0.9 per cent saline, and the populations were counted 6 times in a certified standard hemocytometer. Average dimensions of 25 cells in each group were measured with a calibrated microscope objective.

Respirometry.—The oxygen consumption of 100 mg. (wet weight) of Sarcoma 180 cells in 3 ml. of Krebs-Ringer solution, pH 7.3, plus varying additions of PXP was measured in the usual manner in Warburg vessels over an interval of 60 minutes at 37° C.

Electrophoretic mobility measurements.—The mobilities of tumor cells were determined in a microelectrophoresis apparatus at room temperature with its chamber oriented lateral-
ly as suggested by Hartman, Bateman, and Lauffer (8). Silver-silver chloride electrodes were connected to the microelectrophoresis chamber with 0.5 m KCl bridges. All measurements were made at the upper stationary level by determining the time required for a cell to traverse a predescribed distance, reversing the field, and timing another cell, until the mobilities of ten cells had been observed. The specific conductance was calculated from the conductivity of the cell suspension by means of Maxwell's equation (6). The mobility unit is defined as 1 μ/sec/volt/cm. For all data, ± refers to one standard deviation.

Calculations.—The mobility of the cells was calculated by the expression

\[ \nu = \frac{h q \kappa}{i t} \]  \hspace{1cm} (1)

where \( h \) = distance in microns traversed, \( q \) = cross-sectional area in sq. cm, \( \kappa \) = specific conductance of the solution, \( i \) = current in amperes, \( t \) = time in seconds to traverse \( h \) microns, and \( \nu \) = mobility units.

The charge density was calculated from the modification of the Gouy equation employed by Bateman and Zellner (3) for erythrocytes, assuming an essentially uni-univalent electrolyte environment.

The number of negative charges per mg. of PXP was calculated from the titration curve, assuming complete dissociation and an activity coefficient of 1. At pH 7.3 there are approximately \( 2.3 \times 10^8 \) anions/mg of PXP.

RESULTS

Both Ehrlich and Sarcoma 180 ascites tumor cells exhibit a moderately high net negative charge as judged by electrophoretic mobility. The mean mobility of thirteen different preparations of Sarcoma 180 cells in phosphate buffer of pH 7.3 and 0.172 ionic strength was \(-0.90 \pm 0.14\) mobility units, while that for eight different Ehrlich ascites tumor preparations in the same medium was \(-1.08 \pm 0.15\). The mobility was unaltered by eightfold washing of the cells with isotonic saline, and it remained constant up to 6 hours after collection.

The rate and direction of migration of ascites tumor cells should be markedly dependent upon pH if their cell surfaces are amphoteric. As seen in Charts 1 and 2, Sarcoma 180 and Ehrlich ascites tumor cells are isoelectric at about pH 3.9 and 4.1, respectively, and become electropositive at more acid pH ranges. With the Ehrlich cells, a slight discontinuity was observed between the mobilities in acetate and phosphate buffers. As judged by microscopic examination, the cells were unaffected by the acid media, but above pH 8 some lysis occurred. During the experiments the cells did not significantly alter the pH of the media from pH 8.6 to 6.5 but lowered by 0.1-0.2 pH units the pH of the buffered media in the range from pH 6.5 to 7.6.

Addition of PXP to Sarcoma 180 ascites tumor cells greatly increased their electrophoretic mobility. The mean mobility of these cells was \(-3.02 \pm 0.15\) mobility units in the presence of 0.025 per cent PXP in phosphate buffer of pH 7.3 and ionic strength of 0.178. Assuming that these cells, of a
measured 18.3 μ mean diameter, were spherical, this mobility corresponds to an increase of the net negative charge of the cells of normally about 2900 esu/sq cm to about 9000 esu/sq cm in the presence of PXP. Expressed differently, 1 net negative charge occupied about 2000 Å² normally, whereas four net negatives occupy the same area after adding PXP. Assuming complete dissociation of the phosphate groups of PXP at pH 7.3, 6.5 × 10⁻¹¹ mg. of PXP was bound to each cell.

In Chart 3, the relationship between the concentration of PXP and the net increase in the mobility is shown. The slight effect of low concentrations of PXP indicates that the surface binding of PXP by S-180 cells does not obey a simple adsorption isotherm. The interaction of PXP with Sarcoma 180 ascites tumor cells was influenced both by the ionic strength and by the pH of the medium. In Table 1 is shown the mobility of PXP-treated cells at different ionic strengths. These mobility data, when plotted against the reciprocal of the square root of the ionic strength (a term proportional to the thickness of the ionic double layer), did not extrapolate to zero mobility at infinite ionic strengths. It may be seen from the calculated charge densities that the surfaces of these cells apparently became more electronegative at high ionic strengths.

The effect of the pH of the medium upon the mobility of PXP-treated Sarcoma 180 cells is shown in Chart 1. These cells were more electronegative than the untreated cells over the pH range tested, but their mobility was relatively constant below pH 5.7. The inflection point at the steepest portion of the mobility curve of PXP-treated cells about was 0.4 pH units below the pK of the secondary H⁺ of phosphomonoester groups in this PXP preparation.

Although the mobilities of Sarcoma 180 ascites cells killed with NaCN or NaF were unchanged from the normal, addition of PXP after exposure to these poisons increased the mobility more than

<table>
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<th>Ionic Strength</th>
<th>Ehrlich</th>
<th>S-180</th>
<th>S-180*</th>
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<td>Mobility esu units sq. cm</td>
<td>Mobility esu units sq. cm</td>
<td>Mobility esu units sq. cm</td>
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<tr>
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<tr>
<td>0.0065</td>
<td>-1.51</td>
<td>1310</td>
<td>-1.53</td>
</tr>
</tbody>
</table>

* Incubated 3 hours with 0.02 per cent PXP at the indicated ionic strength.
staining technics, and toluidine blue metachromasia could not be observed. Cell lysis was very pronounced at PXP concentrations of 0.1 per cent or greater and probably accounts for the decreased uptake of PXP$^{32}$ per cell (Chart 4) and for the inhibition of respiration of S-180 cells shown in Chart 5. It will be noted that the concentrations of

PXP required were much greater than those which affect electrophoretic mobility. Here again the extent of inhibition was somewhat dependent upon the population density of tumor cells.

DISCUSSION

The strongly electronegative character of the surfaces of Ehrlich and Sarcoma 180 ascites tumor cells is in keeping with the observation by Ambrose et al. (2) that hamster kidney and liver cells become more negatively charged as they acquire malignant properties during stilbestrol carcinogenesis. A possible relationship between the surface charge of the tumor cell surface and its adhesiveness has been suggested (2, 10). Inasmuch as Robineaux and Bazin (15) were unable to detect any significant differences between the relatively low electrophoretic mobilities of human leukemia cells and their normal counterparts, the surface charge differences observed with experimental tumors may possibly be a phenomenon associated with adaptation of the neoplastic cells to certain growth habits (14).

The electronegativity of Ehrlich and Sarcoma 180 ascites tumor cells at pH 7.3 was nearly as great as that of human erythrocytes (3, 7), but the character of their cell surfaces appears to be entirely different. The isoelectric points of human erythrocytes at about pH 1.9 is indicative of a surface composed of strongly acidic substances such as mucopolysaccharides or cephalin, whereas the isoelectric points of the ascites cells at about pH 4 are much more consonant with the properties of proteins, although lecithin would also be isoelectric at this pH (5). Conceivably, the amphoteric properties of the tumor cells could also be due to adsorbed protein films. The lack of influence of EDTA upon the electrophoretic mobility of the ascites tumor cells makes it unlikely that differences in calcium content of the cell wall (2) are primarily responsible for the increased electronegativity in this case.

In view of the strongly electronegative character of the surfaces of ascites tumor cells, their capacity for binding anionic substances is unexpected. The increased negative charge density of the ascites tumor cells in the presence of higher salt concentration may be due to anion binding or to structural rearrangement of elements of the cell membrane. The expression (3) used to calculate the charge density assumes the cell to have a smooth surface and a large radius of curvature. If increasing salt concentration in an isotonic medium caused a wrinkling of the micro-structure of the surface, changes in radius of curvature

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**CHART 4.** — The effect of various concentrations of PXP$^{32}$ upon the uptake of PXP$^{32}$ per Sarcoma 180 cell, at cell populations of $6.4 \times 10^7$ cells/ml (squares) and $9.8 \times 10^7$ cells/ml (circles). The cells were incubated with PXP$^{32}$ at pH 7.3 and 0.172 ionic strength for 18 hours.

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**CHART 5.** — The inhibition of the respiration of Sarcoma 180 ascites tumor cells by increasing concentrations of PXP at 37°C. The upper (circles) and lower curves (triangles) depict the inhibition with cell populations of $4.4 \times 10^7$ and $5.0 \times 10^7$ cells/ml, respectively.
might also influence the electrophoretic mobility (4).

The increased charge of the tumor cells in the presence of PXP is probably due to surface binding of the polymer rather than to reorientation of preexisting anionic groups within the cell wall. The similarity of the pH mobility curves of PXP-treated cells to the titration curves of PXP indicates that the surface of these cells has essentially the same acidic properties and dissociation constant (K') as PXP itself. However, on the surface of S-180 cells the proportion of weakly anionic groups is considerably greater than was present in PXP. Judging from experiments with PXP\(^{32}\), surface adsorption accounts for only a fraction of the total uptake, and the total uptake of PXP \(^{32}\) by ascites of S-180 cells the proportion of weakly anionic groups is considerably greater than was present in PXP itself. Cyanide and fluoride did not affect the mobility of untreated cells but further increased the mobility of cells in the presence of PXP. Higher concentrations of PXP partially inhibited the respiration and caused some lysis of S-180 ascites tumor cells.

4. From combined electrophoretic and P\(^{32}\) uptake measurements with PXP labeled with P\(^{32}\), surface binding accounts for only a fraction of the total PXP uptake by S-180 cells.

ACKNOWLEDGMENTS

We wish to thank Prof. Henry B. Bull for stimulating consultations and constructive criticisms of this work.

REFERENCES

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