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Electrophoretic Mobility of Ehrlich Ascites Carcinoma Cells Grown *in Vitro* or *in Vivo*[1]

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SUMMARY

The mean electrophoretic mobility of Ehrlich ascites tumor cell populations varies during growth *in vivo*. The mean mobility reaches a maximum one to two days after tumor inoculation and then declines until death of the animals occurs. It is suggested, from measurements of cells after neuraminidase and/or ribonuclease treatment, that changes in the density of peripheral ionogenic groups susceptible to ribonuclease are responsible for the variations in mobility.

INTRODUCTION

It has been reported that changes in cellular electrophoretic mobility may be associated with changes in cellular growth processes (3, 11, 14, 16, 21, 24, 26, 28, 30) and may in some circumstances be related to differences in malignancy and normalcy between "homologous" cells (2, 23). The Ehrlich ascites tumor has been used in many studies of cellular growth processes (8, 15, 22) and has been used extensively in investigations of the nature and properties of ionogenic groups at the surfaces of cells (5, 13, 18, 27, 31, 33–35, 37).

This communication describes experiments designed to investigate possible changes in the electrophoretic mobility of Ehrlich ascites tumor cells during growth in mice or suspension culture.

MATERIALS AND METHODS

Cells

The Ehrlich-Lettré hyperdiploid subline of the Ehrlich ascites carcinoma, obtained from Dr. T. Hauschka at Roswell Park Memorial Institute (RPMI), was used throughout.

Growth in Mice. The tumor was grown in the ascitic form in 6- to 10-week-old HA/ICR ♂ Swiss mice. Tumor cells were removed from mice bearing 7-day-old tumors by means of sterile syringes and were washed twice in Hanks' balanced salt solution (HBSS) pH 7.3–7.9. Normal mice were inoculated intraperitoneally with 1×10^7 cells. The mice died 15–20 days after tumor inoculation. Microscopic examination of washed cells from tumor-bearing mice showed that over 90% of the cells were tumor cells; the remainder were leukocytes. In 0- to 1-day-old tumors the proportion of nontumor cells was 10–20% of the total cell population. Tumor cells extracted from tumor-bearing mice were added to an excess of HBSS. Each mouse was used once only to minimize possible effects of handling and removal of tumor cells on tumor growth. In mice bearing early tumors the mice were killed, and the peritoneal cavities were washed with HBSS to remove the cells.

Growth in Culture. Cells were removed from mice bearing 7-day-old tumors and added to suspension culture flasks containing RPMI medium 1630 (29), supplemented with 5% calf serum, at a final count of approximately 5×10^5 cells/ml. Daily cell counts were made by diluting 1 ml of suspension 1:2 with 1:1000 Trypan blue in HBSS and counting the cells using a hemocytometer. When the proportion of stained cells in the culture reached more than 40%, the cells were washed twice in HBSS and reinjected into normal mice. When the tumor grew in mice the complete procedure was repeated. After several such *in vitro* to *in vivo* passages the tumor cells began to grow in culture as well as in mice. The procedure has been repeated on two separate occasions; in one series *in vitro* growth was established after 9 passages and in the other after 4 passages.

Enzyme Treatment and Preparation for Electrophoresis. Cells removed from mice or culture were washed twice in HBSS and resuspended in HBSS at 37°C at a final cell concentration of $2–20 \times 10^6$ cells/ml. 1-ml aliquots of the cell suspensions, equilibrated at 37°C, were added to either 50 units neuraminidase (Vibrio Cholerae strain Z4, General Biochemical Corp.) in 1 ml HBSS, 0.1 mg ribonuclease (3 × crystallized, Worthington Biochemical Corp.) in 1 ml HBSS, or 1 ml neuraminidase solution mixed with 1 ml ribonuclease solution, and incubated at 37°C for 30 minutes with frequent agitation. Under these conditions a maximum reduction in electrophoretic mobility was reached after 20–25 minutes incubation with either enzyme. Retreatment or prolongation of treatment did not result in any further significant decrease in mobility. Sufficient excess of enzyme was present to allow for possible variations in cell concentration. After 30 minutes treatment by either enzyme, 90% or more of the cells were "viable" as measured by Trypan blue staining. If cells were treated with neuraminidase at its optimum pH 5.5, the cellular viability decreased to 70–80% as assessed by Trypan blue staining.

After incubation the cells were washed once in HBSS, twice in calcium- and magnesium-free Dulbecco and Vogt's phosphate buffered saline (PBS) pH 7.2, and finally suspended in PBS

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at a final cell concentration of 0.5–3 × 10^6 cells/ml for electrophoresis.

Cell Electrophoresis

Cellular electrophoretic mobilities were measured at 25°C ± 1°C in a cylindrical tube apparatus (4). The times for cells to traverse 12.5 or 25 μ in both directions in a 3.2 volts/cm gradient when 1.8 ma of current was flowing were recorded. Between 30 and 205 cells were measured for each cell suspension, and the calculated mobilities were corrected to the viscosity of water at 25°C. In order to check on the reproducibility of the apparatus, the mobility of human Group A Rh+ erythrocytes was determined on each occasion tumor cells were measured. Under the above conditions, the mean mobility of erythrocytes was $-1.026\ \mu\ sec^{-1}\ v^{-1}$ cm (Standard error ± 0.004, in 38 separate experiments). The range in mean mobility of erythrocytes on these 38 occasions was from −0.976 to −1.062.

RESULTS

Cells Grown in Mice. Chart 1 shows the mean mobilities of Ehrlich ascites cells treated with HBSS only. Each point is the mean mobility of a tumor cell population from one mouse. The standard errors of the means ranged from ±0.02 to ±0.04. It can be seen that after an initial rise in mobility, reaching a maximum 1–2 days after inoculation of tumor cells, the mobility steadily decreased until death of the animals occurred. The mean mobility of 1-day-old tumor cells was −1.169 ± 0.027 (standard error; 16 mice), 7-day-old tumor cells −0.989 ± 0.016 (14 mice), 14-day-old tumor cells −0.874 ± 0.022 (11 mice). The mean mobility at zero time is the same as at 7 days, as mice were always inoculated with 7-day-old tumors. The presence of a higher proportion of nontumor cells in young tumors was not considered to be likely to cause the early increase in mobility, as leukocytes have a lower mobility than Ehrlich ascites tumor cells (28).

Chart 1 also shows that there is a marked variation in mean mobility between cell populations from mice with tumors of the same age. These variations are larger than would be expected from experimental variation during the period of study. Analysis of variance showed that cells from 1-day-old tumors showed significantly greater variations in mobility than those from 7-day-old tumors ($P < 0.01$). Due to the large variation in mobility between cells from different mice bearing the same age tumor, a true decrease in mobility during the *in vivo* growth of ascites tumors may not be observed unless a relatively large number of determinations are made. Chart 1 shows an example where the mean mobility of a 7-day-old cell population was higher than that of a 1-day-old tumor.

Chart 2 shows histograms of the mobility of cells at different tumor ages. At the top is the distribution of mobilities of cells from the tumor cell population injected into the mice; below are mobility distributions of cells from this tumor cell popula-

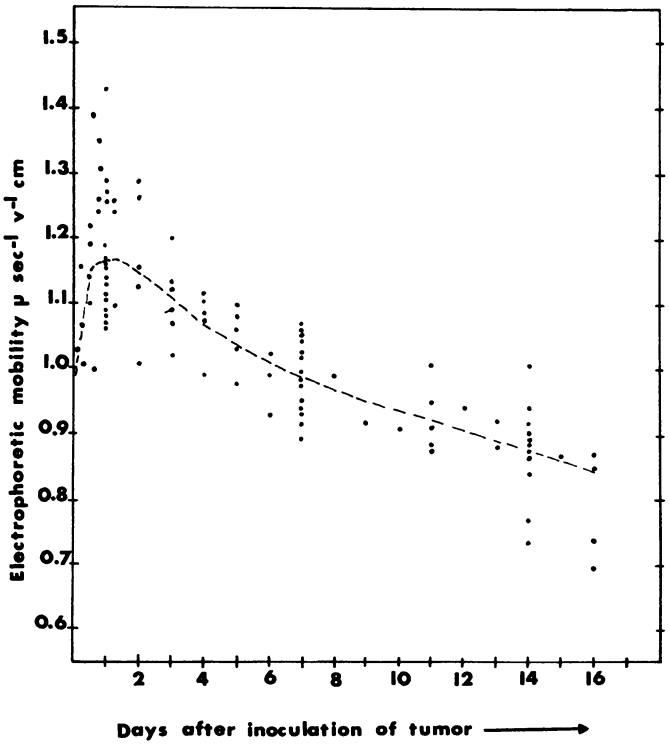

Chart 1. Mean electrophoretic mobility after inoculation of tumor. Each point is mean mobility of a population of Ehrlich ascites tumor cells from one mouse.

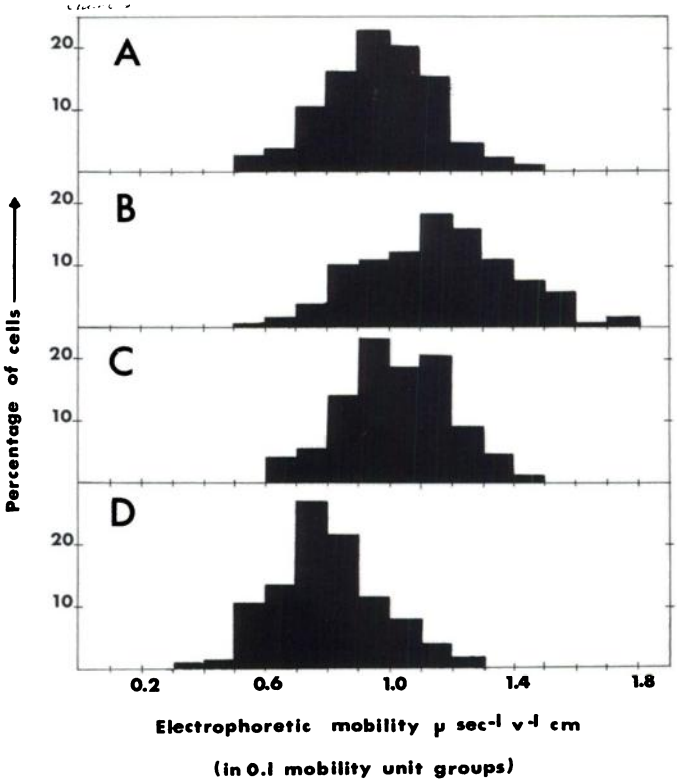

Chart 2. Distribution of mobilities of Ehrlich ascites tumor cells at time of inoculation and 1-, 7-, and 14-day-old tumors. *A*, cells inoculated (1 mouse, 200 cells); *B*, 1 day (4 mice, 204 cells); *C*, 7 days (4 mice, 200 cells); *D*, 14 days (4 mice, 200 cells).

tion at 1, 7, and 14 days respectively. By one day the modal value has moved to a higher value than mobility of the initial inoculum, at seven days the distribution is approximately the same as in the initial inoculum (which was of a 7-day-old tumor), and at 14 days the modal value is less than at 1 or 7 days.

Cultured Cells. During the growth of a tumor cell population *in vitro*, when the mean generation time was 2.2 days, determinations on three different days gave mean mobilities of -0.779 ± 0.027, -0.777 ± 0.030 and -0.803 ± 0.031 respectively. Only 14- to 16-day-old tumor cells grown in mice may have this low mobility (See Chart 1).

Effect of Neuraminidase and/or Ribonuclease. Chart 3 shows the results of an experiment where the effects of neuraminidase and/or ribonuclease on the electrophoretic mobility of Ehrlich ascites tumor cells during growth in mice were determined. Two mice were used at each time from 0 to 1.25 days and at 16 days; three mice were used at other times. The electrophoretic mobilities of 30–100 cells from each mouse were measured after treatment with HBSS, neuraminidase, and/or ribonuclease. The results from different mice were pooled and the means calculated. The vertical bars in the control part of the curve are standard errors of the respective means. The standard errors were calculated for all the other points but are not shown in the chart.

The controls show the same shaped curve as in Chart 1; an initial rise in mobility after inoculation of the tumor with a maximum at 1–2 days was followed by a gradual decrease. The mobility of cells treated with neuraminidase was significantly less ($P < 0.01$) than control cells at all times. The mobility of neuraminidase-treated cells, although less than control cells, followed a curve similarly shaped to that of the controls. The mobility of cells treated with ribonuclease were significantly lower ($P < 0.01$) than controls at all times. However, cells after ribonuclease treatment did not show an initial rise in mobility in the first two days after tumor inoculation. It is probable, taking into account possible experimental variations, that the mobility of cells after treatment with ribonuclease have a constant mobility throughout the growth of the tumor in mice.

In earlier reports (28, 36, 37) when cells were treated with neuraminidase and ribonuclease, the treatments were made separately. However, it was observed that the same reduction in mobility was found when cells were treated with the enzymes mixed in the same solution. After treatment with neuraminidase and ribonuclease together, the mobilities of tumor cells were significantly lower ($P < 0.01$) than controls at all times during tumor growth. Further, the cellular mobilities were significantly less than after treatment with either enzyme alone at all times ($P < 0.01$), except for neuraminidase-treated cells in a 16-day-old tumor ($0.1 > P > 0.05$). After treatment with both enzymes, the cellular electrophoretic mobility remained constant during tumor growth.

Cultured Cells. In an experiment where the mean mobility of control cells was -0.779 ± 0.023, after treatment with neuraminidase the mobility was -0.587 ± 0.020, after treatment with ribonuclease the mobility was -0.536 ± 0.016, and after treatment with both neuraminidase and ribonuclease, the mobility was -0.406 ± 0.005. These results indicated that after treatment with enzymes, the cellular electrophoretic mobility of these cultured cells was similar to enzyme-treated 14- to 16-day-old tumor cells.

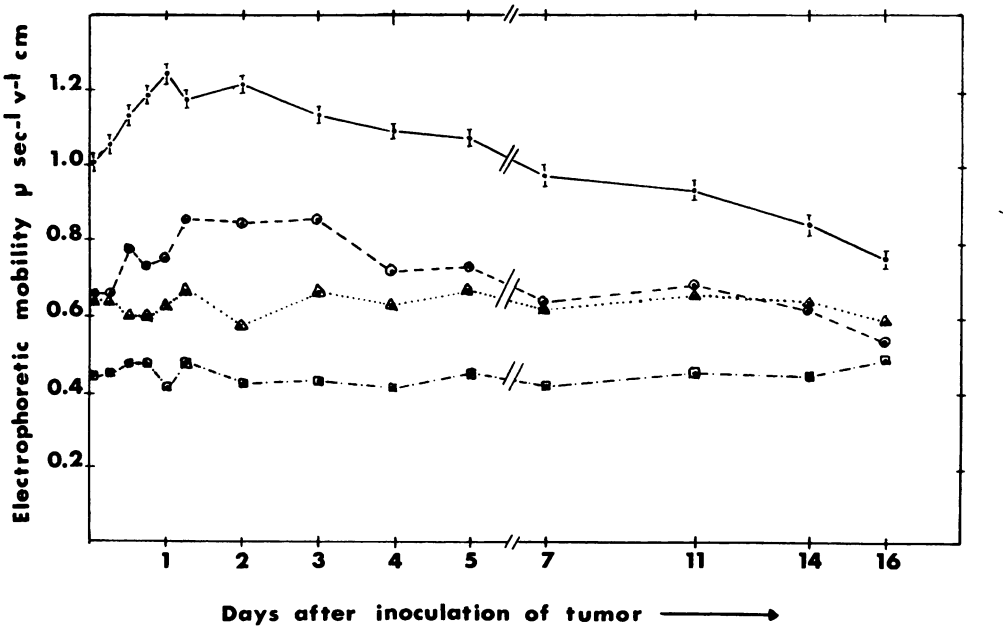

Chart 3. Electrophoretic mobility of Ehrlich ascites tumor cells with or without treatment with neuraminidase and/or ribonuclease. ●———●, controls; ⊙ — — — ⊙, treated with neuraminidase; △ . . . . . △, treated with ribonuclease; ⊡ — . — . — ⊡, treated with neuraminidase and ribonuclease.

Chart 4 shows histograms of mobilities of cells from a 4-day-old tumor and from culture with or without enzyme treatment. Although the variance of the neuraminidase-treated population was slightly less than the variance of the controls, the difference was not significant at the 5% level. The variance of mobility of the ribonuclease-treated cell population was significantly less (at 1% level) than for controls or for those treated with neuraminidase.

DISCUSSION

Measurements of cellular electrophoretic mobility made in physiologic media give an indication of the density of ionogenic groups within 10 Å of the cellular electrokinetic surface (1, 6). It is probable that differences in electrophoretic mobility between different cells, under constant conditions of measurement, are due mainly to differences in cell surface charge density, although the need for caution in extrapolating charge densities from electrophoretic mobility measurements has been emphasized (9, 25).

The results indicate that the mean electrophoretic mobility of Ehrlich ascites cells varies with time after inoculation of tumor, although it has been reported previously that no differences in mobility between 5- to 9-day-old tumors of this type were observable (13, 33). Chart 1 shows that there averages a decrease of about 10% in mobility during this time. However, if observations were made only on 5- to 9-day-old tumors, no conclusions could be drawn concerning the possible occurrence of variations in mean mobility with age of the tumor due to both the small overall change in this time and the large variation in mean mobility among cells from different mice.

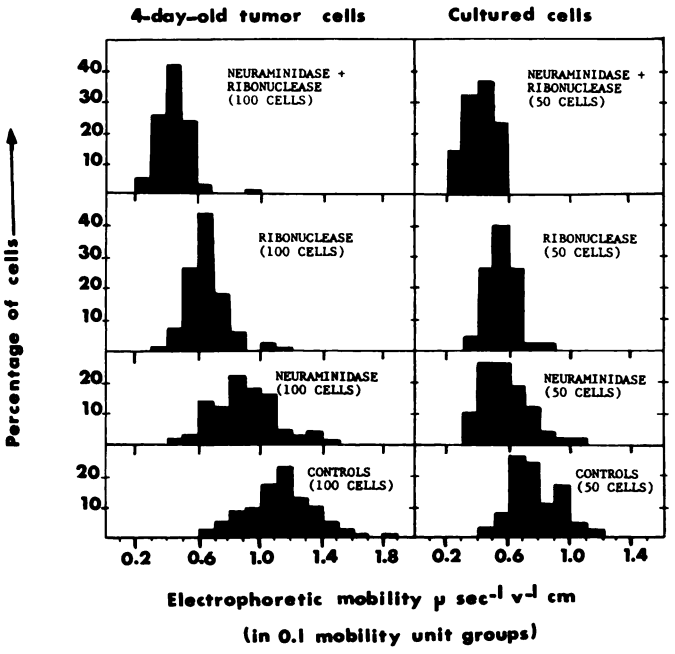

Chart 4. Distribution of mobilities of Ehrlich ascites tumor cells grown in mice or in culture with or without neuraminidase and/or ribonuclease treatment.

Variations in mean mobility of Ehrlich ascites cells extracted from tumors of the same age have been previously noted (5, 27). These variations are much greater than those found among erythrocyte measurements made on different occasions and arise after injection of cells from a common population.

Neuraminidase, which specifically cleaves the glucosidic linkage joining the keto-group of neuraminic acids to *d*-galactose and *d*-galactosamine (20), has been widely used in investigations of the nature of the cell surface. Reduction in cellular mobility after neuraminidase treatment is considered usually to be due to removal of ionized carboxyl groups of neuraminic acid from the electrokinetic surface, although there is not necessarily a direct relationship between the amount of neuraminic acid released and the reduction in electrophoretic mobility (12, 34). All mammalian cells so far studied carry a net negative charge at neutral pH, but only a proportion of them have their electrophoretic mobility changed by ribonuclease (28, 36, 37). Ribonuclease inactivated by the method of Bernard and Stein (7) has no effect on cellular electrophoretic mobility (37), although this inactivated enzyme has overall charge properties very similar to the active enzyme (19). This data suggests that changes in mobility of some cell types, including Ehrlich ascites cells, after ribonuclease treatment are not due simply to charge neutralization after nonspecific adsorption of the enzyme. It is probable that reduction in electrophoretic mobility of some cells indicates the presence of the specific substrate of ribonuclease, secondary phosphate ester linkages of pyrimidine ribonucleosides (10), somewhere within the cell periphery. Although the evidence suggests strongly that changes in electrophoretic mobility of cells after neuraminidase and/or ribonuclease treatment are due to the specific activities of these enzymes, data from enzyme treatment of cells must be interpreted with considerable caution since there are a multitude of factors which may cause changes in mobility (32). The results suggest that ribonuclease reduces the electrophoretic mobility of Ehrlich ascites cells to a constant value, whereas neuraminidase does not. This may indicate that the density of ionogenic sites susceptible to neuraminidase remains relatively constant compared with those susceptible to ribonuclease and that changes in cellular mobility during growth of the tumor are mainly mediated by sites susceptible to ribonuclease. It has been reported (17, 34) that neuraminidase reduces the spread of electrophoretic mobility values in cell populations, but, although neuraminidase reduced the scatter in values slightly in experiments described in this communication, these differences were not significant at the 5% level. However, treatment of cells with ribonuclease caused a marked reduction in scatter of mobilities, suggesting that much of the variation among cells in populations is due to variation in the density of ribonuclease susceptible sites at the cellular electrokinetic surface. The decrease in mobility after treatment with both enzymes was greater than the decrease after treatment with either enzyme separately, possibly indicating loss of both ribonuclease- and neuraminidase-susceptible ionogenic sites from the cell periphery. However, the decrease in mobility after treatment with both enzymes was less than the sum of the decreases after treatment with the enzymes separately and may indicate an association between neuraminidase- and ribonu-

clease-susceptible sites in Ehrlich ascites cells. These results differ from those obtained using RPMI No. 41 cells (derived from a human osteogenic sarcoma) where the effects of the enzymes on electrophoretic mobility were additive (36, 37).

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REFERENCES

1. Abramson, H. A., Moyer, L. S., and Gorin, M. Electrophoresis of Proteins and Chemistry of Cell Surfaces. New York: Reinhold Publishing Co., 1942.
2. Ambrose, E. J., James, A. M., and Lowick, J. H. B. Differences between the Electrical Charge Carried by Normal and Homologous Tumor Cells. Nature, *177:* 576–577, 1956.
3. Band, R. N., and Irvine, B. The Electrokinetic Characteristics of Some Small Amoebae. Exptl. Cell Res., *39:* 121–128, 1965.
4. Bangham, A. D., Flemans, R., Heard, D. H., and Seaman, G. V. F. An Apparatus for Microelectrophoresis of Small Particles. Nature, *182:* 642–644, 1958.
5. Bangham, A. D., Glover, J. C., Hollingshead, S., and Pethica, B. A. The Surface Properties of Some Neoplastic Cells. Biochem. J., *84:* 513–517, 1962.
6. Bangham, A. D., Pethica, B. A., and Seaman, G. V. F. The Charged Groups at the Interface of Some Blood Cells. Biochem. J., *69:* 12–19, 1958.
7. Barnard, E., and Stein, W. D. The Histidine Residue in the Active Centre of Ribonuclease. I. A Specific Reaction with Bromoacetic Acid. J. Mol. Biol., *1:* 339–349, 1958.
8. Baserga, R. Mitotic Cycle of Ascites Tumor Cells. Arch. Pathol., *75:* 156–161, 1963.
9. Brinton, C. C., and Laufer, M. A. The Electrophoresis of Viruses, Bacteria and Cells and the Microscope Method of Electrophoresis. *In:* M. Bier (ed.), Electrophoresis, Theory, Methods and Applications, pp. 427–487. New York: Academic Press, 1959.
10. Brown, D. M., and Todd, A. R. Evidence on the Nature of the Chemical Bonds in Nucleic Acids. *In:* E. Chargaff and J. N. Davidson (eds.), The Nucleic Acids, Vol. 1, pp. 409–446. New York: Academic Press, 1955.
11. Chaudhuri, S., and Lieberman, I. The Surface of the Liver Cell After Partial Hepatectomy. Biochem. Biophys. Res. Commun., *20:* 303–309, 1965.
12. Cook, G. M. W., Heard, D. H., and Seaman, G. V. F. Sialic Acids and the Electrokinetic Charge of the Human Erythrocyte. Nature, *191:* 44–47, 1961.
13. Cook, G. M. W., Heard, D. H., and Seaman, G. V. F. The Electrokinetic Characterization of the Ehrlich Ascites Carcinoma Cell. Exptl. Cell Res., *28:* 27–39, 1962.
14. Cook, G. M. W., Seaman, G. V. F., and Weiss, L. Physicochemical Differences Between Ascitic and Solid Forms of Sarcoma 37 Cells. Cancer Res., *23:* 1813–1818, 1963.
15. Edwards, J. K., Koch, A. L., Youcis, P., Freese, H. L., Laite, M. B., and Donaldson, J. T. Some Characteristics of DNA Synthesis and the Mitotic Cycle in Ehrlich Ascites Tumour Cells. J. Biophys. Biochem. Cytol., *7:* 273–282, 1960.
16. Eisenberg, S., Ben-Or, S., and Doljanski, F. Electrokinetic Properties of Cells in Growth Processes, the Electrophoretic Behaviour of Liver Cells during Regeneration and Postnatal Growth. Exptl. Cell Res., *26:* 451–461, 1962.
17. Forrester, J. A. Microelectrophoresis of Normal and Polyoma Virus Transformed Hamster Kidney Fibroblasts. *In:* E. J. Ambrose (ed.), Cell Electrophoresis, pp. 115–124. London: J. & A. Churchill, Ltd., 1965.
18. Forrester, J. A., Dumonde, D. C., and Ambrose, E. J. The Effects of Antibodies on Cells. II. Changes in the Electrophoretic Mobility of Ascites Tumour Cells Treated With Antibodies and Complement. Immunology, *8:* 37–48, 1965.
19. Glick, G. M., Goren, H. J., and Barnard, E. A. Concurrent Bromoacetate Reaction at Histidine and Methionine Residues in Ribonuclease. Biochem. J., *102:* 7c–10c, 1967.
20. Gottschalk, A. Virus Enzymes and Virus Templates. Physiol. Rev., *37:* 66–83, 1957.
21. Heard, D. H., Seaman, G. V. F., and Simon-Reuss, I. Electrophoretic Mobility of Cultured Mesodermal Tissue Cells. Nature, *190:* 1009, 1961.
22. Klein, G., and Revesz, L. Quantitative Studies on the Multiplication of Neoplastic Cells *in vivo*. I. Growth Curves of the Ehrlich and MCIM Ascites Tumors. J. Natl. Cancer Inst., *14:* 229–277, 1953.
23. Lowick, J. H. B., Purdom, L., James, A. M., and Ambrose, E. J. Some Microelectrophoretic Studies of Normal and Tumour Cells. J. Roy. Microscop. Soc., *80:* 47–57, 1961.
24. Lukiewicz, S., and Kirohoda, W. Electrophoretic Studies on Plant Cells. *In:* E. J. Ambrose (ed.), Cell Electrophoresis, pp. 171–181. London: J. & A. Churchill, Ltd., 1965.
25. Maddy, A. H. The Chemical Organization of the Plasma Membrane of Animal Cells. Intern. Rev. Cytol., *20:* 1–65, 1966.
26. Mayhew, E. Cellular Electrophoretic Mobility and the Mitotic Cycle. J. Gen. Physiol., *49:* 717–725, 1966.
27. Mayhew, E., and Nordling, S. Electrophoretic Mobility of Mouse Cells and Homologous Isolated Nuclei. J. Cell. Physiol., *68:* 75–80, 1966.
28. Mayhew, E., and Weiss, L. Ribonucleic Acid at the Periphery of Different Cell Types and Effect of Growth Rate on Ionogenic Groups in the Periphery of Cultured Cells. Exptl. Cell Res., *50:* 441–453, 1968.
29. Moore, G. E., Sandberg, A. A., and Ulrich, K. Suspension Cell Culture and *in vivo* and *in vitro* chromosome constitution of Mouse Leukemia L1210. J. Natl. Cancer Inst., *36:* 405–413, 1966.
30. Ruhenstroth-Bauer, G., and Fuhrmann, G. F. Sie Negative Überschussladung der Membran von Normalen, Proliferierendon und Malignen Leberzellen der Ratte. Naturforsch., *16b:* 252–254, 1961.
31. Ruhenstroth-Bauer, G., Fuhrmann, G. F., Kubler, W., Rueff, F., and Munk, K. Zur Bedentung der Neuraminsäuren in des Zellmembran fur das Wachstum Maligner Zeller. Z. Krebsforsch., *65:* 37–43, 1962.
32. Seaman, G. V. F., and Cook, G. M. W. Modification of the Electrophoretic Behaviour of the Erythrocyte by Chemical and Enzymatic Methods. *In:* E. J. Ambrose (ed.), Cell Electrophoresis, pp. 48–65. London: J. & A. Churchill, Ltd., 1965.
33. Simon-Reuss, I., Cook, G. M. W., Seaman, G. V. F., and Heard, D. H. Electrophoretic Studies on Some Types of Mammalian Tissue Cell. Cancer Res., *24:* 2038–2043, 1964.

34. Wallach, D. F. H., and De Perez Esandi, M. Sialic Acid and the Electrophoretic Mobility of Three Tumour Cell Types. Biochim. Biophys. Acta, *83:* 363–366, 1964.
35. Wallach, D. F. H., and Eylar, E. H. Sialic Acid in the Cellular Membranes of Ehrlich Ascites Carcinoma Cells. Biochim. Biophys. Acta, *52:* 594–596, 1961.
36. Weiss, L., and Mayhew, E. The Presence of Ribonucleic Acid Within the Peripheral Zone of Two Types of Mammalian Cell. J. Cell. Physiol., *68:* 345–360, 1966.
37. Weiss, L., and Mayhew, E. Ribonucleic Acid Within the Cellular Peripheral Zone and the Binding of Calcium to Ionogenic Sites. J. Cell. Physiol., *69:* 281–292, 1967.

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