Cellular Electrophoretic Mobility and the Mitotic Cycle

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ABSTRACT The electrophoretic mobility of RPMI No. 41 cells grown in suspension, parasynchronized by double thymidine blocking and cold shock, is reported. No. 41 cells have a higher electrophoretic mobility during the mitotic peak phase than at other times in the mitotic cycle. Treatment of parasynchronous cells by neuraminidase reduces the mobility to the same value irrespective of the stage of the cells in the mitotic cycle. The higher electrophoretic mobility of cells in mitotic peak phase is probably due to a higher surface charge density at this time, possibly caused by a higher concentration of ionized neuraminic acid carboxyl groups at the hydrodynamic shear layer. The mobility of nonsynchronous rapidly and slowly growing cells differs; neuraminidase reduces their mobility by proportionately similar amounts. The results suggest that the differences in mobility between rapidly and slowly growing cells cannot be accounted for exclusively by differences in the amount of neuraminic acid groups at the shear layer.

Eisenberg et al. (8) noted greater variability in the electrophoretic mobilities of rapidly growing liver cells compared with those growing more slowly. This observation could suggest that at different stages during the mitotic cycle cells may have different electrophoretic mobilities. In a preliminary communication (13) some change was reported in the mobility of cultured cells parasynchronized by double thymidine blocking. In this communication further studies are reported in which another synchronization technique was used, and the effect of neuraminidase on the mobility of synchronous cells was studied.

MATERIALS AND METHODS

Stock Cell Culture RPMI No. 41 cells derived from a human osteogenic sarcoma (16) cultured in suspension at 37°C in RPMI medium 906 (16), supplemented by 5% calf serum, were used throughout. The density of the cell suspension was maintained at 200 to 250 $\times$ 10^3 cells/ml; the glucose level at 1200 mg/liter and pH at 7–7.4.

In exponential growth No. 41 cells have a mean generation time of 19 $\pm$ 1 hr

and mitosis (late prophase to the end of telophase) takes 30 to 35 min. DNA synthesis time, determined by the autoradiographic method of Baserga and Lisco (4), is 5.9 ± 0.3 hr.

Parasynchronous Cell Culture

Two methods to induce parasynchronous growth were used. (*a*) double thymidine blocking (5, 18, 20), and (*b*) cold shock (17).

(A) DOUBLE THYMIDINE BLOCKING To stock cultures which had been growing exponentially for at least 5 generations, thymidine was added to give a final concentration of 0.01 M and after 14 hr the cells were washed twice and resuspended in fresh medium at 37°C. 10 hr later fresh thymidine was added to the cultures to give a final concentration of 0.01 M and 14 hr later the cells were again washed twice and resuspended in fresh medium at 37°C. Zero hour was the time of release from the second thymidine treatment.

(B) COLD SHOCK Cultures which had been growing exponentially for at least 4 generations were allowed to grow until the cell concentration rose to over 4×10^5 per ml. The cell concentration was then reduced to half by adding an equal volume of fresh medium at 2–4°C, and the temperature of the cultures was reduced to, and maintained at 4°C with continuous stirring. After 1 hr culture vessels were replaced in a 37° incubator; this was zero hour.

Cell samples were removed at intervals after release from cold shock or thymidine treatment for determination of cell number and viability, mitotic index, and electrophoretic mobility.

Cell Number and Viability

1 ml samples were withdrawn from the culture vessels and mixed with 0.5 ml of 1% trypan blue in Hanks' solution. Counts, accurate to ±5%, were made of the number of stained ("dead") and unstained ("alive") cells using a hemocytometer. In a properly maintained cell culture, there were never more than 3% dead cells, but in some experiments in which exponentially growing cultures were deliberately deprived of fresh medium to slow down the rate of cell division, the proportion of dead cells rose to over 10%.

Mitotic Index

A drop of the cell suspension on a slide was mixed with an equal volume of 1% orcein in glacial acetic acid and a cover slip was pressed down on this. The number of cells in mitosis (from late prophase to the end of telophase) in a random sample of 2000 cells was determined. Determinations were accurate to ± 10%.

Preparation for Electrophoresis

Cell suspensions containing 10×10^6 cells were removed from culture and were centrifuged for 5 min at 500 g, and washed twice in Hanks' balanced salt solution. Untreated control cells were resuspended in 5 to 7 ml of half-strength Hanks' in 5% sucrose (pH 7.6 ± 0.2) and incubated at 25°C. In the "neuraminidase" series, the control cells were resuspended in 2 ml calcium-saline (0.145 M NaCl; 0.005 M

$CaCl_2$; 3×10^{-4} M $NaHCO_3$; pH 7.2 ± 0.2), and the enzyme-treated cells were resuspended in 1 ml calcium-saline plus 1 ml 100 units/ml neuraminidase in calcium-saline (1 volume *Behringwerke* stock neuraminidase, free of protease, aldolase, phospholipase, and lipase activity (31), 500 units/ml diluted with 4 volumes calcium-saline). This concentration of neuraminidase was calculated to be in excess of the amount needed to liberate all of the neuraminic acid present in 10×10^6 cells. After 30 min incubation at 37°C the cell suspensions, with or without neuraminidase, were washed once in Hanks' solution by centrifuging for 5 min at 500 g and resuspended in half-strength Hanks' solution in 5% sucrose.

Electrophoresis

After equilibration for 10 min at 25°C, the times taken for individual cells to move 25 μ at 25 ± 1°C, 3.16 v/cm, in a cylindrical chamber electrophoresis apparatus (3) were measured and their electrophoretic mobilities were calculated. In each determination at least 50 cells were measured and usually over 100. Measurements could be completed within 1 hr after removal of cells from the culture or incubator.

Oxygen Utilization

Measurements were made of the oxygen utilization of cells for 3 hr after treatment with neuraminidase, using a Warburg apparatus (24).

RESULTS

Fig. 1 shows the electrophoretic mobilities, mitotic indices, and cell concentrations in four experiments; in three parasynchronization by cold shock and in one, double thymidine blocking were used.

The electrophoretic mobility of cells varies with time after release from the synchronizing agent, and is at a maximum when the percentage of mitotic cells is highest. The mobility at this time (mitotic peak phase) is significantly higher (t-test, $P < 0.01$) than after release from the synchronizing agent or after cell division. In experiment 1, in which most readings were taken, the increase in mean mobility starts about 4 to 8 hr after release, rises to a peak 10 to 14 hr after release, and falls sharply 14 to 16 hr after release. In all experiments, the increase in mean mobility of cells at mitotic peak phase was 14 to 21% higher than that of cells at zero time. Neuraminidase reduced the mobility to 50 to 60% of the initial value in unsynchronized cultures. In parasynchronous cultures, neuraminidase treatment also caused a reduction in electrophoretic mobility which remained constant irrespective of the time after release from cold; t-tests showed no significant difference between samples at any time after release in a single experiment. It can be seen that when the initial electrophoretic mobility was low (experiment 1), the mobility of neuraminidase-treated cells was low and when the initial mobility was high (experiment 2), the mobility of neuraminidase-treated cells was high.

It was found that the oxygen utilization of cells after treatment with neuraminidase was not significantly different from that of untreated cells up to 3 hr after treatment.

In parasynchronous cultures the mitotic peak never occurred at exactly

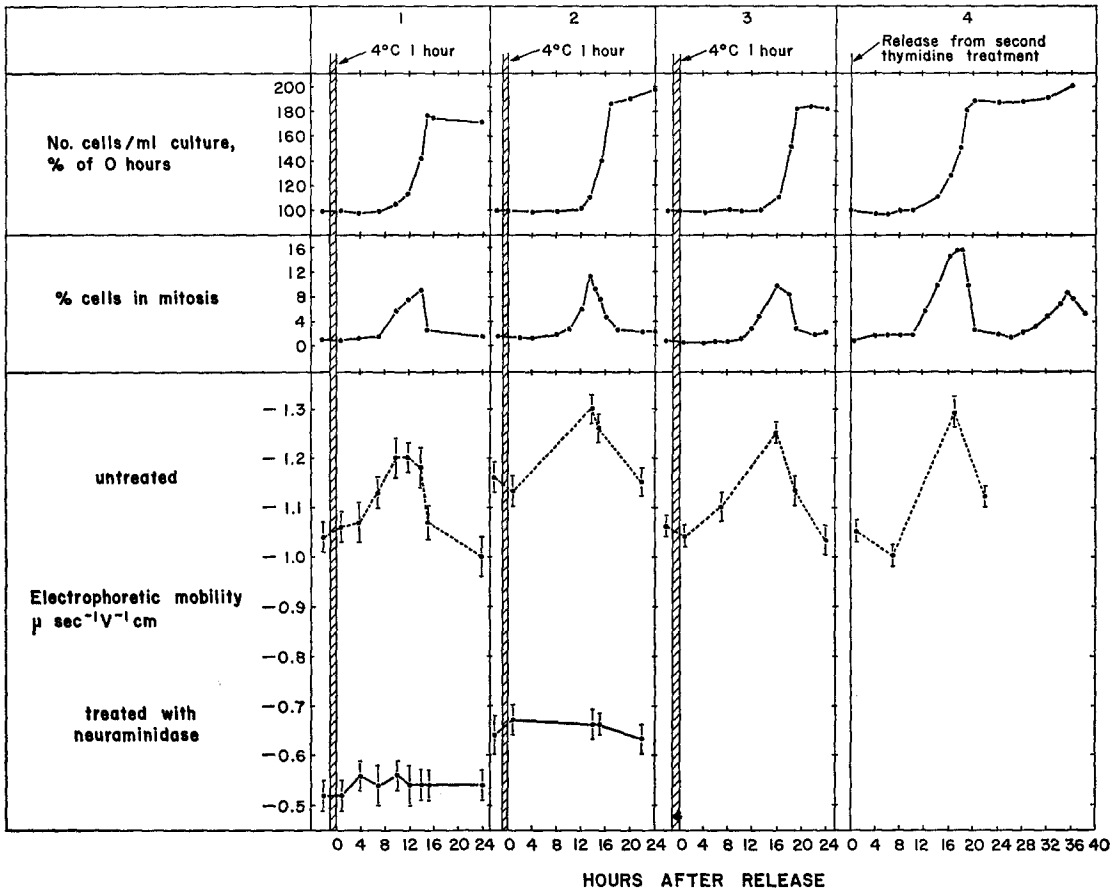

FIGURE 1. Changes in cell number, % dividing cells, and electrophoretic mobility of No. 41 cells, with time after release from synchronizing agent. Vertical lines represent ±1 SE.

the same time in different experiments. In cultures synchronized by cold shock the peak occurred 13 to 16 hr after release, whereas in thymidine-synchronized cultures the peak occurred 14 to 18 hr after release. The maximum number of cells in mitosis varied from 9 to 10% for cells after cold shock to 14 to 15% after treatment with thymidine. However, the cell division rate was not much greater for cells treated by thymidine than it was compared with those treated by cold shock. The degree of synchronization (9) varied between 50 to 80%, the highest value being for the thymidine-treated

cells. Calculations of mitotic time by integration of mitotic index curves showed that mitosis took a normal time, i.e. 30 to 35 min, in cold shock–treated cultures whereas thymidine-treated cells took at least 50% longer. Cultures grew well for at least 8 generations after release from thymidine indicating that this deviation from normal behavior does not seem to permanently damage the cells. It will be noted from Fig. 1 that in one "division cycle" the cell population does not double, but that the increase in cell count varied between 75 to 90%, indicating that 82 to 95% of the cells divided.

Table I shows the mobilities of cells from either rapidly growing or slowly growing cultures which contained 85% of cells "viable" by the parameter

TABLE I

ELECTROPHORETIC MOBILITIES OF RAPIDLY GROWING AND SLOWLY GROWING NO. 41 CELLS WITH AND WITHOUT NEURAMINIDASE TREATMENT

Cell type	Mobilities $\mu sec^{-1}v^{-1}$ cm ± SE		Percentage decrease in mobility of cells treated with neuraminidase
	Untreated	Neuraminidase-treated	
No. 41 rapidly growing	−1.09±0.03(102)*	−0.63±0.02(103)	42
No. 41 slowly growing	−0.86±0.02(110)	−0.49±0.03(100)	43

* Numbers in parentheses indicate number of cells measured.

of trypan blue uptake. It can be seen that the mobility of the nongrowing cells is lower than that of growing cells, both for untreated and neuraminidase-treated samples. It can be seen also that the ratios of mobilities of neuraminidase-treated cells and untreated control cells are approximately the same for both types of culture.

DISCUSSION

If the observations described in this report have a general validity for mammalian cells, they may be relevant in studies of interactions among cells. The possible role of surface charge in this field has been pointed out in several reviews (1, 7, 22, 29).

For particles the size of cells: Electrophoretic mobility (μ) is given by the Helmholtz-Smoluchowski equation (2),

$$\mu = \frac{\zeta\epsilon}{4\pi\eta} = \frac{\sigma\chi}{\eta}$$

Where ζ = zeta potential.

ϵ = dielectric constant of the medium at the electrophoretic shear layer.

η = viscosity of the medium at the electrokinetic shear layer.

σ = charge density of the particle surfaces.

χ = effective thickness of the ionic double layer surrounding the surface.

Under constant experimental conditions, as described in this report, the assumption that ϵ, η, and χ remain constant can be made, and thus the electrophoretic mobility of the cells is directly proportional to the charge density at the cell surface and is independent of cell size.

If the above assumptions are valid, the measurements of electrophoretic mobility described in this communication would indicate that RPMI No. 41 cells have the highest charge density during the mitotic peak phase when the cells present are in late interphase (G_2), mitosis, and early interphase (G_1). It appears probable, however, that the cells having the highest charge density are those in G_2 and/or mitosis, as after division the mobility falls sharply.

The net negative charge at the cell surface is thought to be partly due, in most cells studied, to the charged carboxyls of *N*-acetylneuraminic acid (6, 10, 26, 28). Treatment of most cells by neuraminidase reduces their electrophoretic mobility but there is not necessarily a direct relationship between charge reduction and sialic acid release (27). However, the fact that after neuraminidase treatment the mean mobility of parasynchronous cells is the same at any time during the mitotic cycle suggests that the increased charge density of cells at the mitotic peak phase may be due to higher concentration of the ionized carboxyl groups of *N*-acetylneuraminic acid in the hydrodynamic shear layer.

Consideration of the changes occurring in cell division may give an indication of the mechanism of charge density changes. During interphase, the volume of actively growing cells increases so that just before or during mitosis, a maximum is reached, after which the cell divides into two daughters. Considering the parent and daughter cells as spheres, either their total volumes or their surface areas must change during division. Two possibilities exist for change of surface area or volume during division:

1. If the total surface area in parent and both daughter cells remains the same, combined volume of the daughter cells would be 30% less than that of the parent cell. However, studies on different cell types have indicated that each daughter cell has approximately 50% of the volume of the parent cell (12, 19). If these results hold for cultured cells, then volume changes would be ruled out.

2. If, on the other hand, the volume of the parent cell is the same as the combined volumes of the daughter cells, the combined surface area of the daughter cells would be approximately 28% greater than that of the parent cell (33). The hypothesis can be advanced that cell surface *N*-acetylneuraminic acid is synthesized during later interphase and/or mitosis and the

resulting higher density of ionized carboxyl groups causes a higher electrophoretic mobility during mitosis; after division a drop in charge density occurs, possibly caused by stretching of the cell surface. The results presented in this paper would support this hypothesis although they do not prove it.

The groups which cause the higher electrophoretic mobility may well cause decreased deformability prior to or during mitosis by conferring extra rigidity on the underlying cell periphery (11, 30, 31). It is therefore of interest to note that Mitchison and Swann (15) have observed decreased deformability expressed as increased rigidity in sea urchin eggs during cleavage. The higher charge and consequent reduction in deformability of cells before and/or during mitosis could also possibly account for the observed separation of mitotic cells from surfaces, and/or from one another (23).

After neuraminidase treatment the percentage reduction in electrophoretic mobility of unsynchronized rapidly growing and slowly growing cells is the same. This possibly indicates that the difference in charge density of untreated growing and nongrowing cells is due to proportionately equal loss of positively and negatively charged groups. Also it is possible that change in charge may be associated with change of metabolic rate (32). In a preliminary communication (13), it was shown that a few cells with more than twice the mean electrophoretic mobility of unsynchronized cells were present in mitotic peak phase samples, possibly suggesting that at one specific time in mitosis cells may carry a particularly high charge.

Both methods described in this communication for inducing parasynchronous growth, synchronize cells by altering their normal growth pattern, and as pointed out by Mitchison (14) these alterations could change cellular properties, including their electrokinetic character. However, although the methods used in the present work to obtain synchronization are different, the results are similar, and it is therefore unlikely that the synchronization techniques in themselves are producing the changes described here.

Although it would appear from the present results that the electrophoretic mobility changes only during G_2 and/or mitosis, it would be unwise to assume that charge-dependent properties remain constant at other times in the mitotic cycle. Electrophoretic data only give information on cellular net surface charge and not on surface organization and cinemicrography shows that cells not only have different surface activities during the mitotic cycle, but also one cell can exhibit several different types of activities at the same time. Probably the cell surface organization varies not only during the mitotic cycle but also from place to place on the cell surface.

Average parameters of various surface properties, based on studies on whole cell populations which are random with respect to mitosis, may be misleading. However, studies on synchronized populations will enable more

precise observations to be made on the changing properties of cells during the mitotic cycle, such as susceptibility to drugs and radiation (21, 25).

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REFERENCES

1. ABERCROMBIE, M., and AMBROSE, E. J., *Cancer Research*, 1962, **22**, 525.
2. ABRAMSON, H. A., MOYER, L. S., and GORIN, M., Electrophoresis of Proteins, and Chemistry of Cell Surfaces, New York, Reinhold Publishing Corp., 1942.
3. BANGHAM, A. D., FLEMANS, R., HEARD, D. H., and SEAMAN, G. V. F., Nature, 1958, **182**, 642.
4. BASERGA, R., and LISCO, E., *J. Nat. Cancer Inst.*, 1963, **31**, 1559.
5. BOOTSMA, D., BUDKE, L., and VOS O., *Exp. Cell Research*, 1964, **33**, 301.
6. COOK, G. M. W., HEARD, D. H., and SEAMAN, G. V. F., *Nature*, 1961, **191**, 44.
7. CURTIS, A. S. G., *Biol. Rev.*, 1962, **37**, 82.
8. EISENBERG, S., BEN-OR, S., and DOLJANSKI, F., *Exp. Cell Research*, 1962, **26**, 451.
9. ENGELBERG, J., *Exp. Cell Research*, 1961, **23**, 218.
10. FORRESTER, J. A., AMBROSE, E. J., and MACPHERSON, J. A., *Nature*, 1962, **196**, 1068.
11. GOTTSCHALK, A., *Nature*, 1960, **186**, 949.
12. HIRAMOTO Y., *J. Exp. Biol.*, 1958, **35**, 407.
13. MAYHEW, E., and O'GRADY, E. A., *Nature*, 1965, **207**, 86.
14. MITCHISON, J. M., *Exp. Cell Research*, 1957, **13**, 244.
15. MITCHISON, J. M., and SWANN, M. M., *J. Exp. Biol.*, 1954, **31**, 461.
16. MOORE, G. E., MOUNT, D., TARA, G., and SCHWARTZ, W., *Cancer Research*, 1963, **23**, 1735.
17. NEWTON, A. A., and WILDY, P., *Exp. Cell Research*, 1959, **16**, 624.
18. PETERSEN, D. F., and ANDERSON, E. C., *Nature*, 1964, **203**, 642.
19. PRESCOTT, D. M., *Exp. Cell Research*, 1955, **9**, 328.
20. PUCK, T. T., *Science*, 1964, **144**, 565.
21. SINCLAIR, W. K., and MORTON, R. A., *Biophysic. J.*, 1965, **5**, 1.
22. STEINBERG, M. S., The Problem of Adhesive Selectivity in Cellular Interactions in Cell Membranes in Development, (M. Locke, editor), New York, Academic Press, Inc., 1964.
23. TERASIMA, T., and TOLMACH, L. J., *Exp. Cell Research*, 1963, **30**, 344.
24. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F., Manometric Techniques, Minneapolis, Burgess Publishing Co., 1964.
25. WALKER, I. G., and HELLENER, C. W., *Cancer Research*, 1963, **23**, 734.
26. WALLACH, D. F. H., and EYLAR, E. H., *Biochim. et Biophysica Acta*, 1961, **52**, 594.

27. WALLACH, D. F. H., and DE PEREZ ESANDI, M. V., *Biochim. et Biophysica Acta*, 1964, **83**, 363.
28. WEISS, L., *Nature*, 1961, **191**, 1108.
29. WEISS, L., *Biochem. Soc. Symp.*, 1962, **22**, 32.
30. WEISS, L., *J. Theoret. Biol.*, 1964, **6**, 275.
31. WEISS, L., 1965, *J. Cell Biol.*, **26**, 735.
32. WEISS, L., 1966, *J. Nat. Cancer Inst.*, in press.
33. WOLPERT, L., *Internat. Rev. Cytol.*, 1960, **10**, 163.