Changes in the metabolic state of a cell that permit (and perhaps trigger) mitosis are suggested from a variety of observations (see general discussions of the premitotic state: references 5, 7, 13). A list of such premonitory indications of mitosis should probably include increase in nuclear volume, loss of cellular water, cortical and endoplasmic viscosity changes partly or wholly related to calcium shifts, transient availability of protein sulfhydryl groups, as well as some sort of filling of an energy store.

With mammalian cells, increased cell electrophoretic mobility has been repeatedly associated with cell division (3, 6, 11, 17). Mayhew (12) has recently shown that in parasynchronous cultures of human osteosarcoma cells (RPMI No. 41) the transient elevation of mean cell electrophoretic mobility occurring just before, and during the division wave is related to neuraminidase-susceptible groups on the cell surface. Since electrophoretic mobility is a function of surface charge density (rather than total charge) and since surface sialic acid is well known as a participant in electrophoretic mobility of many kinds of cells (1, 2, 4, 21), Mayhew's observations raise an interesting question (discussed in some detail in his paper, as well as in a recent paper by Weiss, 24): does the transient charge density elevation indicate a transient elevation of the density of surface sialic acid molecules, or does it, instead, relate to a change in the spatial or charge exposure relationship of the same molecules to the electrokinetic shear layer?

Previous work in this laboratory (8) indicated that changes in total sialic acid per cell were associated with changes in mean cell volume when parasynchronous Chinese hamster ovary (CHO) cells were followed through a division wave. Assays of randomly growing cells showed that two-thirds of the total sialic acid was located on the cell surface, one-third being bound to intracellular molecules. These conclusions were reached on the basis of Warren assays (23) of materials released from either intact cells or broken cells by means of neuraminidase treatment or mild acid hydrolysis. It was conceivable, therefore, that electrophoretic mobility changes related to cell division might involve fluctuations of intracellular and surface sialic acid even though the shift was probably too rapid to invoke a mechanism involving synthesis de novo and release (9, 18). The present report deals with measurement of surface sialic acid as a function of cell surface area in parasynchronous CHO cells released from thymidine block (15).

**METHODS AND RESULTS**

Methods for assay of surface sialic acid (as N-acetyleneuraminic acid) and for determination of mean cell surface area and procedures for production and sampling of parasynchronous CHO cultures were the same as previously described (8, 9). As far as could be determined by optical microscopy, the cells had completely smooth surfaces and were nearly spherical in shape, except during the actual period of cytokinesis.

Briefly, CHO suspension cultures were resuspended in fresh medium after 9 hr of block in medium containing 10 mM thymidine. Samples were withdrawn thereafter every hour for 12 hr for determination of cell count (by electronic particle counting), mean cell surface area (computed from mean cell volume as determined by a
100-channel pulse-height analyzer used in conjunction with a modified Coulter aperture, and surface sialic acid: i.e., sialic acid released with neuraminidase under conditions in which no release of intracellular sialic acid occurred (8, 9). The latter determination involved Warren assays of material released from intact cells with neuraminidase, with the appropriate precautions being taken to avoid interference by other colored compounds (23).

Further evidence for the idea that these assays did, in fact, measure sialic acid of surface membranes was obtained from assays of material released by mild acid hydrolysis of whole cells and membrane pellets from cells that were either untreated or neuraminidase-treated before analysis (Table I).

Fig. 1 represents data from a typical experiment, which indicate that the burst of cell division occurring between 5 and 9 hr after release was correlated with decrease in mean cell volume occurring at the same time. It is also evident, when surface sialic acid is expressed as molecules per μ² surface area, that no sudden shifts in this function occur

### TABLE I

<table>
<thead>
<tr>
<th>Treatment of intact cells</th>
<th>Hydrolysis of intact cells</th>
<th>Hydrolysis of membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>11.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>3.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Molecules per cell X 10⁻⁸.
† Membrane pellets were prepared by freeze-thaw rupture of cells 4 times, centrifugation at 105,000 g for 2 hr, washing the pellet with balanced salt solution, and recentrifugation at 105,000 g for 2 hr. Hydrolysis to release terminal sialic acid groups was done in 0.1 N H₂SO₄ at 80°C for 1 hr.

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**Figure 1** Surface sialic acid per unit cell surface area, mean cell volume, and cell count of a CHO suspension culture released from thymidine block at time 0. ○, sialic acid molecules (×10⁻⁸) per μ² crude cell surface area; □, mean cell volume, μ²; and ▲, cells per ml × 10⁻¹.
relative to the period of maximum cell division nor, for that matter, throughout the entire experiment. Furthermore, when the same data are expressed as neuraminidase-released sialic acid per equal aliquot of culture, the points describe a smoothly increasing line, with no indication of the sudden changes in slope that would be expected if a sudden change in surface sialic acid had occurred in relation to the burst of cell division. A gradual increase in surface density of sialic acid, as illustrated in Fig. 1, has been observed on a number of occasions. The significance of this increase is unknown at present, but the data are clearly inconsistent with a transient mass increase related to division wave.

DISCUSSION

Since neuraminidase-susceptible sialic acid is thought to represent exclusively terminal sialic acid molecules on oligosaccharide chains (16) with free carboxyl groups of low pK (free sialic acid has a pK of 2.6—see reference 20), it might be assumed that there would be a simple 1:1 relationship between surface sialic acid molecules and surface negative charge. That such is generally not the case has been clearly shown by several groups (14, 21, 22). Even with mammalian red cells, in which sialic acid accounts for the bulk of negative charge and the relation of charge and molecules of sialic acid is roughly linear, it is not 1:1 (4). Both Eylar and his coworkers (4) and Seaman and Uhlenbruck (19) have discussed possible reasons for these discrepancies.

The transient, neuraminidase-susceptible mobility shift observed by Mayhew could, in general, be explained by any or all of the following mechanisms: (a) a change in surface density of terminal sialic acid molecules; (b) a change in effective in situ pK of the carboxyl groups of terminal sialic acid molecules; or (c) a change in topology of surface sialic acid molecules in relation to electrokinetic surface. Our data, that no surface density shift occurs on a mass basis, are essentially complementary to those of Mayhew and tend to exclude the first possible mechanism listed above.

It is well to bear in mind, however, the assumptions upon which this conclusion is based. The parameter surface sialic acid per unit area involves a mean volume estimate, an assumption of smooth spherical shape, and a colorimetric assay of a supernatant fluid removed following enzymatic treatment of living cells. The validity of volume spectroscopy and of the Warren assay in these conditions seems well established (8, 23; E. C. Anderson and D. F. Petersen, in preparation). That neuraminidase does not enter the cells also seems certain from results cited above, as well as recent results of Marcus et al. (10). The question of the nature and extent of the error introduced by the calculation of surface area from volume thus seems the most important reservation on the conclusion. However, this reservation also seems less serious, since the equal-aliquot data that do not involve area calculations are consistent with the mass per area data.

Thus, two types of conclusions can be reached. With greater certainty it can be said that the cellular electrokinetic surface, whose charge density determines the electrophoretic mobility, and the surface that bears sialic acid molecules accessible to neuraminidase are not identical. Then, with some reservations, it seems likely that the transient elevation of electrophoretic mobility prior to cell division is due to conformational or terminal complex changes of sialo-glycolipids or sialoglycopeptides resident on the cell surface throughout the process.

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REFERENCES