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ELECTROKINETIC PROPERTIES OF NUCLEAR SURFACES

A COMPARISON OF NUCLEI FROM NORMAL AND REGENERATING RAT LIVER

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SUMMARY

1. Studies of various isolation procedures for nuclei were found to yield nuclear surfaces with significantly different electrokinetic properties. Citric acid-extracted nuclei have surface properties similar to those of the intact liver cell; citric acid–sucrose-extracted nuclei also have similar characteristics although these may be masked by variable irreversible DNA adsorption especially when using low ionic strength media for their isolation or electrophoretic examination. Sucrose-extracted nuclei displayed considerable electrokinetic instability consistent with the gradual desorption of surface cytoplasmic components.

2. A comparison of nuclei from normal and regenerating liver revealed no difference in their surface electrokinetic properties despite the probable adsorption of DNA at the nuclear surface in both groups when citric acid–sucrose-isolated nuclei were examined.

3. In no instance was sialic acid demonstrable on the nuclear surface.

INTRODUCTION

LILLIE reported in 1903 (ref. 1) that anodic migration of isolated nuclei was greater in those containing more nucleic acid. Recent studies by LIEBERMAN and co-workers on regenerating liver[2-6] have shown an increase in anodic electrophoretic mobility of both intact cells and isolated nuclei. They deduced there is an increase in surface neuraminate which is related to the synthesis of RNA and functional protein.

Sialic acid has been demonstrated in the plasma membrane of normal rat-liver cells[7,8] although it apparently does not contribute to the electrophoretic properties[9]. However, PATTERSON AND TOUSTER[10] could not detect any sialic acid in the nuclear fraction from normal rat liver.

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In the present work some electrophoretic characteristics of the nuclear surface from normal and regenerating liver cells are documented and effects of nuclear isolation procedures on electrokinetic properties of nuclear surfaces are demonstrated.

METHODS AND MATERIALS

All studies were performed on the livers of adult hooded rats (140–175 g body wt.) of an inbred strain. Partial hepatectomy was performed[11] allowing a 67–72% removal. After perfusion of the portal vein with cold physiological saline containing 0.5% disodium EDTA, intact liver cells were isolated by a method employing sequential grinding with sterile sand, filtration through cheese-cloth, and repeated washings in saline at graded centrifugal speeds. Rat red blood cells were studied in the same manner as human red blood cells[12].

Nuclei were isolated in three different media. The method of REES AND ROWLAND[13] was employed to obtain nuclei in 0.25 M aqueous sucrose; that of DOUNCE[14] for nuclei in citric acid and nuclei were also isolated using the method of KISHIMOTO AND LIEBERMAN[2] employing 0.1 M citric acid. All nuclear fractions were examined by light and electron microscopy. Nuclei were prepared for electron microscopy by fixation in 5% glutaraldehyde for 2–3 h, and post-fixed in 1% osmium tetroxide at 4° for 1 h. After washing and dehydration in graded ethanol, embedding was carried out in Epon 812 (ref. 15) and in a modified Maraglas[16]. Silver sections were mounted on bare 300–400-mesh copper grids, stained by the combined lead citrate[17] and uranyl acetate[18] techniques and examined in a Siemens Elmiskop I at 60 kV.

Electrophoretic mobilities were determined at 25° using a modification of the cylindrical cell described by SEAMAN[19], the small volume (1 ml) needed for measurement was particularly advantageous. A solution of standard isotonic saline (0.15 M aq. NaCl made $3 \cdot 10^{-4}$M with respect to $NaHCO_3$, pH 7.2 ± 0.2) was used for reversible pH–mobility studies[12]. Electrophoretic mobilities were corrected to the viscosity of standard isotonic saline at 25°. Aliquots of saline-washed nuclei were treated with neuraminidase (Behringwerke) as described[20] and the supernatant fluids analysed for sialic acid[21]. Similar aliquots of saline-washed nuclei were incubated at 37° for 30 min with 1.0 mg/ml deoxyribonuclease (2 × crystallized, Nutritional Biochemicals Corp.), 1.0 mg/ml ribonuclease (crystalline, Nutritional Biochemicals Corp.) in isotonic saline buffered to pH 7.5 with bicarbonate; control aliquots were incubated in the absence of enzyme.

RESULTS

In Fig. 1 a comparison of rat erythrocyte and intact liver cell surfaces suggests the essentially polyanionic nature of the red-cell surface and protein-like character of the liver cell surface, which has an isoelectric point at approx. 4.0. In Fig. 2 a comparison of three nuclear extraction procedures on the resulting pH–mobility curves of nuclei is shown. The sucrose and citric acid curves have protein characteristics[22], the citric acid curve closely approximating the intact liver cell curve (see Fig. 1). The high anodic mobilities and low isoelectric point in the citric acid–sucrose nuclei preparations may arise from DNA adsorbed to the peripheral region.

Citric acid and citric acid–sucrose-extracted nuclei showed no electrokinetic

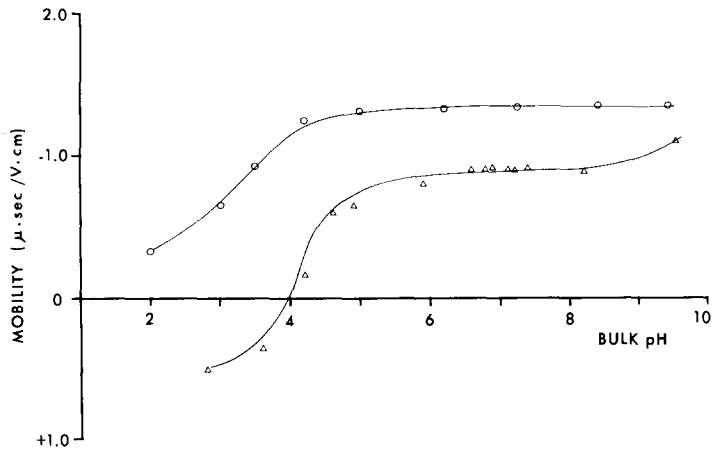

Fig. 1. The effect of pH on the electrophoretic mobility of normal rat cells in 0.15 M NaCl. Each point represents the mean value of ten mobility readings. ○, red cells; △, intact liver cells.

evidence of irreversible surface structural changes following exposure to low and high pH solutions; sucrose-extracted nuclei, on the other hand, failed to show satisfactory pH–mobility reversibility. Citric acid- and citric acid–sucrose-extracted nuclei also displayed reproducible electrokinetic behaviour when stored for 12 h at 4°; sucrose-extracted nuclei were unstable, however.

Fig. 3 demonstrates absence of contribution of sialic acid to the electrokinetic surface of (citric acid-extracted) normal and regenerating nuclei. Similar results were obtained using citric acid–sucrose-extracted nuclei.

Fig. 4 demonstrates the following: (1) Citric acid–sucrose-extracted nuclei from normal and regenerating liver have similar mobility curves with high anodic mobilities and a low isoelectric point. (2) Treatment with deoxyribonuclease reduced the anodic

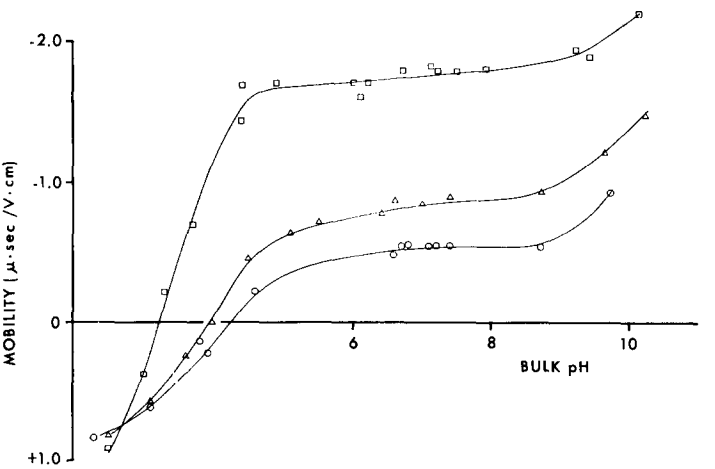

Fig. 2. pH–mobility relationships of isolated nuclei, in 0.15 M NaCl, from normal rat liver. ○, sucrose-extracted nuclei; △, citric acid-extracted nuclei; □, citric acid–sucrose-extracted nuclei.

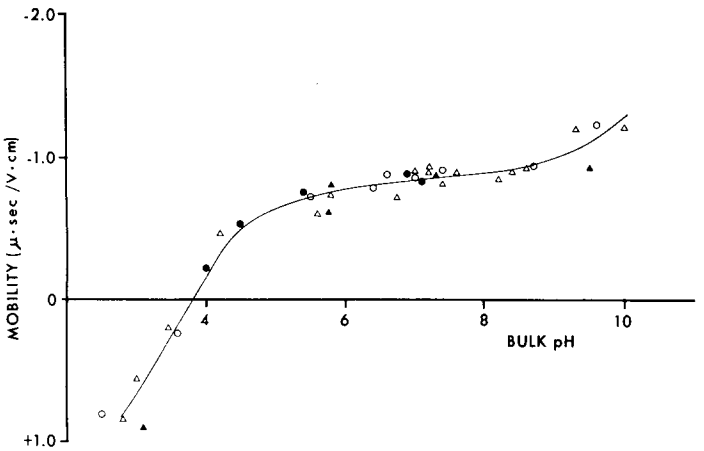

Fig. 3. pH–mobility relationships of citric acid-extracted nuclei (in 0.15 M NaCl) treated with neuraminidase. ○, normal nuclei control; ●, normal nuclei treated with neuraminidase; △, regenerating nuclei; ▲, regenerating nuclei treated with neuraminidase.

mobility of both normal and regenerating nuclei over the pH range 5 to 10 and also raised the isoelectric point. (3) Deoxyribonuclease-treated nuclei have pH–mobility curves that approximate to those found for untreated normal and regenerating nuclei extracted with citric acid (see Fig. 3).

Electron micrographs of the nuclear fractions both from normal and regenerating livers revealed freedom from contamination. The limiting membrane appeared to consist of a single osmiophilic layer rather than the bilamellar structure characteristic of osmium-fixed nuclei within intact liver cells, or those isolated solely in sucrose.

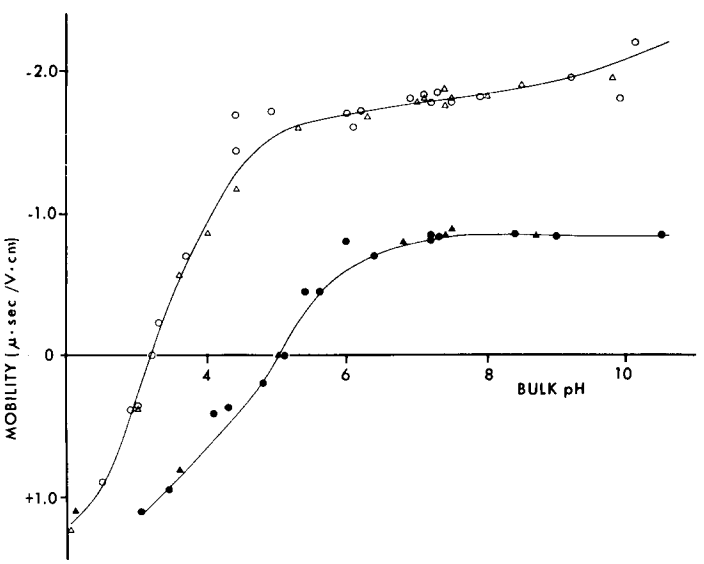

Fig. 4. pH–mobility relationships of citric acid–sucrose-extracted nuclei (in 0.15 M NaCl) treated with deoxyribonuclease. ○, normal nuclei; ●, normal nuclei treated with deoxyribonuclease; △, regenerating nuclei; ▲, regenerating nuclei treated with deoxyribonuclease.

The amount of sialic acid released by neuraminidase per ml per 10^9 nuclei was below the limit of detection of the method. Ribonuclease was without effect on the electrokinetic properties of nuclei.

DISCUSSION

The electrokinetic charge of a cell or subcellular particle depends on either the distribution of ionogenic groups which are components of the molecular architecture of the envelope and/or its interaction with the surrounding medium. If the nuclear membranes are porous to the ions of the electrical double layer then electrophoretic methods may permit an analysis of the molecular anatomy up to distances of about 8 Å into the membrane in 0.15 g-ions/l ionic strength electrolytes, this distance corresponding to the effective thickness of the electrical double layer. However, if the peripheral region is a dilute aqueous gel, the penetration depth of electrophoresis may depend upon the ease of hydrodynamic drainage rather than on the double layer thickness.

Electron microscope observations have shown that the nuclear envelope is bilamellar and the outer membrane continuous in places with endoplasmic reticulum. Sucrose-extracted nuclei retain the outer membrane, but treatment with citric acid removes this surface leaving the inner layer intact[23]. These findings were confirmed in the present study and extended to demonstrate loss of the outer membrane using the citric acid–sucrose isolation method. Nuclei isolated in citric acid gave protein-like pH–mobility curves at various ionic strengths that were fully reversible and closely similar to those of intact liver cells[9]. In addition, the failure of ribonuclease, deoxyribonuclease and neuraminidase to affect the electrophoretic properties of such nuclei is further support for the suggestion that citric acid preparations of nuclei gave a "clean" surface.

Nuclei isolated in sucrose gave pH–mobility curves which were unstable with respect to time. At high or low pH values desorption of cytoplasmic contaminants appeared to occur such that the electrokinetic properties of the sucrose nuclei tended irreversibly towards those of the citric acid nuclei.

Nuclei isolated by the citric acid–sucrose method had a high anodic mobility and low isoelectric point (Fig. 2) suggestive of the presence of a strongly acidic component such as neuraminate or phosphate at the electrophoretic surface. Neuraminidase treatment of such nuclei produced no change in their electrokinetic properties nor was detectable sialic acid released. However, treatment of the citric acid–sucrose-isolated nuclei with deoxyribonuclease resulted in a pH–mobility curve (Fig. 4) approximating that for nuclei extracted solely in aqueous citric acid. The change in the surface characteristics of the citric acid–sucrose-extracted nuclei produced by the action of added deoxyribonuclease may have arisen from the degradation of DNA present at the electrophoretic surface of the nucleus. This surface DNA may have arisen from intranuclear DNA which had leaked out during the isolation procedure and become adsorbed under the attendant conditions of low ionic strength. The adsorption of many substances is likely to be enhanced under conditions of low ionic strength since, as can be deduced from Debye–Hückel theory, the work required to separate charged molecules increases with decreasing ionic strength of the ambient fluid. This view is supported by the adsorption of hemoglobin by red cells[24] and the

irreversible adsorption of alkaline phosphatase by nuclei[25]. In this connection, BEINERT[26] has shown, using an isotopic method, that the nuclear content of cytochrome *c* arose from its readsorption under conditions of low ionic strength.

Examination of citric acid and citric acid–sucrose-isolated nuclei from regenerating liver failed to reveal any electrokinetic properties that differed from nuclei isolated from normal liver, thus our findings do not support the observations[2] that regenerating nuclei exhibit an increase in anodic mobility compared with normal nuclei. It is apparent from the likely presence of DNA on normal and regenerating nuclear surfaces following exposure to low ionic strength media that electrophoretic data should be interpreted with caution. KISHIMOTO AND LIEBERMAN[2] and CHAUDHURI AND LIEBERMAN[27] carried out their measurements in low ionic strength phosphate-buffered sucrose and their differences between nuclei from normal and regenerating liver could have arisen from differential adsorption of intranuclear leakage products at the interface; it further emphasizes the effect of isolation procedures on the subsequent chemical composition of nuclei[28,29].

The nature of the ionogenic groups responsible for the electrokinetic charge of "clean" citric acid-extracted nuclei has not been established but it appears from the pH–mobility curves, that protein carboxyl groups may be responsible.

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