density gradient. It can also be used as a diagnostic or investigative tool to indicate variation in cell parameters in clinical or research material. Such variations would be meaningful in studying ovulating periods, hormonal inbalances, and/or the beginning of cancerous conditions in clinical material which contain both normal and abnormal cells. It can also be used to physically separate groups of cells or microorganisms for continued study and growth, the monitoring of cell cultures, and the separation of cell generations in synchronous cultures[5].

Zusammenfassung. Dichtegefälle-Ultrazentrifugation, verbunden mit Photopolymerisation von Akrylamid, ist geeignet um lebende, unverletzte Zellen unbeweglich in Bänder zu trennen, insofern sie sich punkto Gewicht, Volumen, Dichte oder Beweglichkeit im Dichtegefälle unterscheiden. Verschieden ernährte Hefezellen-Gruppen (*Candida albicans*) zeigten verschiedene Bandenverteilungen.

C. G. Saltarelli, R. Stearns and O. S. Hum

Roswell Park Memorial Institute, Department of Health, 666 Elm Street, Buffalo (New York 14203, USA), and State University of New York, School of Engineering, Buffalo (N.Y. 14203, USA), 1 August 1969.

[5] Supported by the School of Engineering, State University of New York at Buffalo; the Samuel Higby Camp Foundation of Jackson, Michigan; and the Roswell Park Memorial Institute in Buffalo, New York.

Calcium Ion Binding to Blood Cell Surfaces

This communication reports the uptake of calcium ions by the peripheral region of the human red blood cell (RBC) and polymorphonuclear leucocyte (PMN), before and after neuraminidase treatment, as studied by cell electrophoresis.

Cell electrophoresis was performed as described previously[1]. Solutions containing different molarities of calcium at constant osmolarity, ionic strength and pH were prepared by dilution of a $0.0483\,M$ aqueous calcium chloride solution made $0.145\,M$ with respect to sucrose, with a $0.145\,M$ aqueous sodium chloride solution. PMNs were separated using the method of Carruthers[2]. Separated PMNs were finally washed twice with 50 vol. of standard saline at room temperature ($100 \times g$; 7 min). Neuraminidase treatment of the erythrocytes and PMNs was carried out as described by Seaman and Uhlenbruck[3]. Supernatant fluids from neuraminidase treated cells and controls were analyzed for sialic acid[4,5]. Normal and neuraminidase treated RBCs and PMNs were washed once in, and re-suspended in, each of the aqueous calcium chloride solutions at pH 7.2 ± 0.2 and examined by electrophoresis.

Using Stern's adsorption model[6] in which the adsorption of ions is assumed to be essentially monomolecular on to widely spaced immobile non-interacting sites it can be deduced

$$\text{that} \qquad \Delta\sigma_{Ca} = 2en_{Ca} = \frac{2e\,N_a}{1+exp\left(-\frac{\overline{\Delta G}_{Ca}/kT)}{\chi_{Ca}}\right)}, \qquad \text{(I)}$$

where $\Delta\sigma_{Ca}$ is the decrease in electrokinetic charge density of the cell in a solution of C g-ions per liter of calcium ions, χ_{Ca} mole fraction of calcium ions where $\chi_{Ca} = C/55.6$, n_{Ca} number of calcium ions adsorbed per cm^2, e the electronic charge, N_a the number of sites available for adsorption (number of anionogenic groups per cm^2), $\overline{\Delta G}_{Ca}$ electrochemical free energy of adsorption of calcium ions, k the Boltzmann constant and T the absolute temperature.

$$\text{Now let} \qquad exp\,(\overline{\Delta G}/kT)/55.6 = K$$

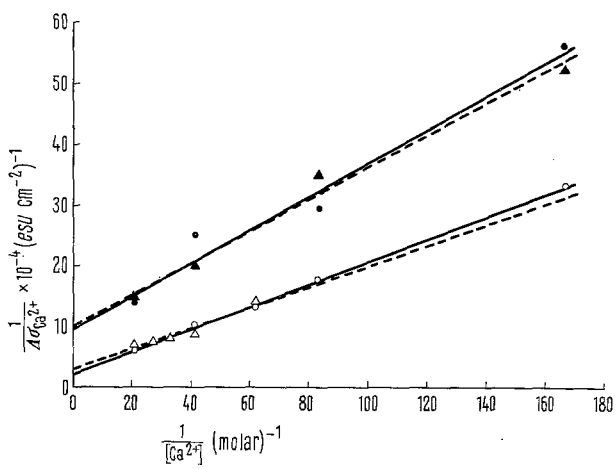
$$\text{then} \qquad \Delta\sigma_{Ca} = \frac{2eN_aCK}{1+CK} \qquad \text{(II)}$$

a standard form for a Langmuir adsorption isotherm, or in linear form,

$$\frac{1}{\Delta\sigma_{Ca}} = \frac{1}{2eN_a} + \frac{1}{C}\,\frac{1}{2eN_aK} \qquad \text{(III)}$$

If calcium ion binding proceeds according to this mass action mechanism, then, the plot of $(1/\Delta\sigma_{Ca})$ versus $(1/C)$ will be a straight line. N_a and $\overline{\Delta G}_{Ca}$ may be evaluated from the intercept with the ordinate and the slope respectively.

The number of anionic sites per square centimeter of cell surface was calculated from the electrophoretic mobilities by use of the Gouy equation for uni-valent electrolytes and converted to a number of sites per cell assuming an area of 1.63×10^{-6} cm^2 for the RBC and 2.84×10^{-6} cm^2 for the PMNs[7]. The Figure shows the plots of $(1/\Delta\sigma_{Ca})$ versus



Calcium ion binding plots for normal (○—○) (Slope 0.185×10^{-4}, intercept $2.1 \pm 0.4 \times 10^{-4}$) and neuraminidase treated (●—●) (Slope 0.276×10^{-4}, intercept $9.5 \pm 2.8 \times 10^{-4}$) erythrocytes and also normal (△---△) (Slope 0.173×10^{-4}, intercept $2.7 \pm 1.2 \times 10^{-4}$) and neuraminidase treated (▲----▲) (Slope 0.261×10^{-4}, intercept $9.9 \pm 2.2 \times 10^{-4}$) polymorphonuclear leucocytes. Symbols represent experimental points.

Calcium ion binding data for human polymorphonuclear leucocytes and red blood cells

System	Anionic sites per cell $\times 10^6$	No. of binding sites for Ca^{2+} per cell $\times 10^6$	$\overline{\Delta G}$ Kcal mole^{-1}	Charge reversal concentration (M) Ca^{2+}
RBCs	12.5	8.09 + 1.92 − 1.30	− 3.82 + 0.10 − 0.13	0.300
Neuraminidase treated RBCs	3.90	1.79 + 0.74 − 0.41	− 4.47 + 0.16 − 0.20	
PMNs	17.8	10.9 + 0.93 − 0.34	− 4.01 + 0.22 − 0.36	0.284
Neuraminidase treated PMNs	7.14	2.99 + 0.83 − 0.53	− 4.53 + 0.12 − 0.15	

($1/C$) for normal and neuraminidase treated human RBCs and PMNs. The intercepts of these lines with the ordinates, from which N_a was calculated, were obtained analytically from linear regressions of the points obtained at high calcium ion concentrations. The slopes of the lines were taken from the regression equations and the electrochemical free energies of adsorption calculated from these values using equations (II) and (III) (Table). The charge reversal concentrations for calcium ions were found from the abscissae of the points whose ordinates were equal to the reciprocal of the control charge densities. For these points, $\sigma = 0$ and the predicted value of C which would cause this zero net charge is taken as the charge reversal concentration of calcium.

The principal electrophoretic effect of an increasing concentration of calcium ions on blood cells may be ascribed to binding of the ions to the charge determining regions in the cellular periphery with a resultant decrease in the electrokinetic charge. Approximately 65% of the anionic sites of the RBCs and about 60% of those of the PMNs are occupied by calcium ions at maximum binding, whereas only about 45 and 40% respectively of the sites available after neuraminidase treatment are bound by calcium ions. Although fewer calcium ions are bound to the neuraminidase treated cells they are bound more strongly than to normal cells. In order to account for the observed changes in calcium ion binding after neuraminidase treatment the presence of at least 3 types of anionic configuration in the peripheral regions of the cells is required, 2 of which bind calcium ions. The binding sites comprise both neuraminate ions and a set of unidentified sites which bind calcium more strongly than neuraminate ions. A third set of charge configurations with little or no affinity for calcium is also present. These differences in calcium ion binding need not imply the presence of different ionogenic groups as the sites may differ only in their accessibility to the calcium ions. The electrochemical free energies of adsorption of calcium ions for the normal RBCs and PMNs given in the Table represent mean values for both types of site present whereas the values for the neuraminidase treated systems represent only those sites with a greater affinity for calcium ions than the neuraminate ions.

The existence of a charge reversal concentration of calcium ions for both normal RBCs and PMNs implies the adsorption of 1 calcium ion per singly charged ionogenic group for a significant number of the sites in the cellular periphery. Such an adsorption process is consistent with the low electrokinetic charge density and wide average separation between each anionic site. The absence of charge reversal concentrations for calcium ions for the neuraminidase treated RBCs and PMNs is an indication of the high proportion of sites which do not bind calcium ions significantly in comparison with those which bind calcium ions more strongly than neuraminate ions.

The results presented show that the calcium ions are bound to at least 2 types of site in the peripheral regions of RBCs and PMNs which differ significantly in their intrinsic affinities and that in addition there are anionic sites at which little or no calcium ion binding occurs[8].

Zusammenfassung. Neue Erkenntnisse über die quantitativen Zusammenhänge von fixierten, negativen Ladungen an Zellmembranen und ihren Interaktionen mit Kalzium-Ionen. Die elektrophoretische Analyse der Bindung von Ca-Ionen an menschliche polymorphkernige Leukozyten und rote Blutkörperchen deutet die Anwesenheit von mindestens 3 verschiedenen anionischen Ladungskonfigurationen in der peripheren Zone der Zellen an. Die zwischen den Ca-Ionen und den Oberflächen-Anionen existierenden elektrochemischen freien Bindungsenergien sind relativ schwach; daraus folgert, dass die Ca-Ionen nur schlechte Komponenten für eine direkte interzelluläre Überbrückung darstellen.

G. V. F. SEAMAN, P. S. VASSAR[9] and M. J. KENDALL[9]

Division of Neurology, University of Oregon Medical School, Portland (Oregon 97201, USA), and Department of Pathology, University of British Columbia, Vancouver 9 (B.C., Canada), 7 *July 1969.*

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9 Address: Department of Pathology, University of British Columbia, Vancouver 9 (B.C. Canada).