ELECTROPHORESIS OF STEROLS

II. ERGOSTEROL

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Although such a large literature on the electrophoretic behavior of cell surfaces exists (1), our knowledge of the ζ-potentials of many of the important constituents of these interfaces is meager. Partly for this reason, attempts to identify surface components from the electrokinetic properties of living cells have met with difficulty. These uncertainties have to a large extent been removed by the numerous investigations now available on protein systems (1, 2). Among the lipids, however, only lecithin and cholesterol have been investigated to any extent (3). The previous work on cholesterol has been summarized in an earlier paper (4). Partly as a comparison and partly because of its own importance in cell activities, it was decided to investigate ergosterol from an electrokinetic standpoint.

Methods

Ergosterol of a high degree of purity was secured from Dr. Charles E. Bills. This contained (in addition to the one molecule of water of crystallization) about 4 per cent of α-dihydroergosterol (5). Due to the limited quantity available further purification was not attempted. The material consisted of snow-white crystals and showed no signs of being contaminated with oxidation products. While in our possession it was always stored at 0°C. in the dark. To prepare suspensions the method used for cholesterol (4) was employed. This consisted in grinding the sample for an hour with pure ice in an agate mortar at −10°C. The resultant powder was kept in the dark at −10°C. until needed. No changes were noted on storage at this temperature.

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This pulverized sterol when dissolved in chloroform exhibited the characteristic red to blue reaction of ergosterol with trichloracetic acid (6). With mercuric acetate in nitric acid the color change was from transient red to yellow. This is indicative that the material was not changed or oxidized by the treatments employed to produce suspensions (7). As needed, suspensions of the powdered material were made in distilled water. Acetate buffers of constant ionic strength \( \mu = 1/150 \) were used. A modified Northrop-Kunitz microelectrophoresis apparatus was employed in the determination of mobilities. The rest of the technique concerning buffer preparation, electrophoresis, quinhydrone pH measurement, etc., is described in previous papers (4, 8, 9). Following the suggestion of Abramson (1), temperatures were corrected by multiplying the velocities at the temperature \( t \) by the fraction \( \eta_t / \eta_{25} \) where \( \eta \) is the viscosity of water at \( t \) and 25°.

EXPERIMENTAL

The experimental results are shown in Fig. 1. It was found that ergosterol becomes isoelectric at pH 3.1 instead of pH 3.2, as previously found for cholesterol (4). The material was also more variable in its behavior. As before, reversal in sign was noted but not to any great extent. The smooth line running through the points is the curve fitted by the use of Langmuir's adsorption equation to the data for cholesterol. The excellent agreement between the two sets of data shows that within the limits of experimental error the two sterols are identical in electrophoretic behavior. The extrapolated dash line is merely to indicate sign reversal.

Several explanations might be advanced to account for this similarity: (1) the presence of an ampholyte as an impurity common to both; (2) the occurrence of sufficient ergosterol as a natural contaminant of the cholesterol; (3) a slight dissociation of OH⁻ ions from each sterol; (4) preferential adsorption of H⁺ or OH⁻ ions. The first possibility seems to be excluded upon consideration of the high melting points of the cholesterol preparations and the extensive purificatory treatments which they received (4). As shown by Rosenheim and Webster (10) and others, ergosterol occurs naturally in preparations of cholesterol. Two factors militate against the possibility of ergosterol causing the \( \xi \)-potential of cholesterol, (1) the small quantities
reported—amounting to less than 1 part per thousand of cholesterol (10, 11) and (2) the method of preparing the suspension by crushing which gives no chance for the ergosterol to leave the crystal and coat the surface. Further experiments were undertaken to test this assumption.

Cholesterol from spinal cords of cattle which had been carefully purified by saponification (4) was also refluxed with norit after the procedure of Rosenheim and Webster (10). After filtration, the colorless alcoholic solution was allowed to crystallize (m.p. 148.5-149° corr.), vacuum-dried, and made into a suspension by grinding. No changes in velocity or isoelectric point were found. Although Bills, Honeywell, and MacNair (11) have shown that this procedure does not remove every trace of ergosterol, a change in behavior would be expected if ergosterol were the causative agent. Furthermore, the Rosenheim and Callow (7) trichloracetic acid test for ergosterol was negative with all of the author's preparations of cholesterol.

Both sterols have a single OH radical. The possibility of ionization as a base at this group to the extent needed to produce charge reversal seems slight. Incidentally, Remesow (12) has found that particles of the cholesterol esters of stearic, oleic, and palmitic acids are negative at all pH values.

The fact that the two sets of data are both fitted by the same Langmuir equation (4)
ELECTROPHORESIS OF STEROLES. II

\[ V = \frac{\alpha \beta [\text{OH}]}{1 + \alpha [\text{OH}]} \quad (1) \]

(where \( \alpha = 8.14 \times 10^9 \), \( \beta = 1.57 \), and \( V \) is the velocity in \( \mu \text{sec./volt/cm.} \)), which has also been employed with success by Abramson and Müller (13) and Abramson (1) in their studies of the charge of "inert" surfaces, is evidence for preferential adsorption of \( \text{H}^+ \) or \( \text{OH}^- \) ions, as the basis of the reaction.

Such adsorption would tend to change \( \sigma \), the effective surface charge per unit area. By the use of the Gouy and Helmholtz equations (4, 13, 14) it can be shown that if \( \kappa \), the reciprocal of the effective thickness of the double layer, remains constant and \( \kappa r \gg 1 \) (where \( r \) is the radius of the particle), \( \sigma \) becomes directly proportional to \( V \) for small values of \( V \). Hence \( V \) can be used in equation (1) instead of \( \sigma \).

Activities should more properly be used in this equation instead of "concentrations" but since uncertainty still exists concerning the correct value for the calomel electrode (\( E_0 \)) and the liquid junction potential (\( E_0 \)) involved in pH measurement (15), both the experimental points and the theoretical curve in Fig. 1 are expressed in terms of the Sørensen standards proposed by Clark (16), with the assumption that \( p\text{OH} = -\log_{10}C_{\text{OH}} \). If a value of \( E_0 \) which will give \( p\text{OH} = -\log_{10}C_{\text{OH}} \) (so that the values of \( a_{\text{OH}} \) will nearly equal values of the mean ionic activity of the acetic acid in the solution) is finally agreed upon, the value for \( \beta \) in equation (1) will remain the same and the only change necessary to replace \( \text{pH} \) by \( p\text{OH} \) in Fig. 1 will be a slight shift of the abscissa to the left for a distance of about 0.04 \( \text{pH} \) unit. The \( C_{\text{OH}} \) is used instead of the \( C_{\text{H}} \) simply for convenience.

As stated before, the isoelectric point was not included in the calculations but the extrapolated curve ran through it.

The mere fact that the Langmuir equation fits one set of data does not prove an adsorption mechanism but the goodness of fit for both of these different crystals seems to strengthen the evidence. It seems significant that Abramson (17) has found that the levorotary forms of the insoluble amino acids, cystine, tyrosine, and aspartic acid, all reverse their sign of charge between \( \text{pH} \) 2.3 and 2.5. The present

**1** This can only be done if the error introduced by substituting \( f(V) \) for \( \sinh f(V) \) is negligible.
LAURENCE S. MOYER

communication should be of interest in the analysis of the surfaces of latex particles (8, 9, 18).

SUMMARY

The electrophoretic behavior of powdered ergosterol crystals is identical with that previously found for cholesterol within the limits of experimental error. Evidence for an adsorption hypothesis is presented to explain this phenomenon.

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BIBLIOGRAPHY
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