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Electrophoretic Behavior of the Papilloma Virus Protein.*

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In 1937 a specific heavy protein was isolated[1] by differential ultracentrifugation from infectious extracts of cottontail-rabbit papillomas.[2] Purified by several cycles of alternate low- and high-speed ultracentrifugation the material sediments in the analytical ultracentrifuge with the sharp boundary characteristic of a single molecular species and with a sedimentation constant of $S_{20}^{\circ} = 265 \times 10^{-13}$ cm sec^{-1} $dynes^{-1}$. Studies on infectivity,[3] complement-fixing capacity,[4] and neutralization[5] with specific immune sera have demonstrated a high degree of uniformity in the biological behavior of the protein and its intimate relation to the papilloma virus. In the present work, further evidence of the homogeneity of the macromolecular protein is furnished by examination of its electrophoretic behavior.

The studies were made on about 150 mg of protein freshly prepared from 350 g of warts from many cottontail rabbits, extracted and purified by 3 ultracentrifugal cycles by the routine procedure previously described.[1] The purified product was dissolved in buffer solution consisting of 0.05 M sodium chloride and 0.05 M sodium veronal-sodium acetate to give a solution of 0.1 ionic strength and adjusted to the required pH with NaOH or HCl. Studies were made on dialyzed solutions at protein concentrations of 4.5 to 2.7 mg per cc. The instrument† employed was of the Tiselius[6] type. Movement of the protein boundary in the 11 cc cell under the influence of constant electric potential at 0°C and changes

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1 Beard, J. W., Bryan, W. R., and Wyckoff, R. W. G., *J. Infect. Dis.*, 1939, **65**, 43.

2 Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

3 Bryan, W. R., and Beard, J. W., *J. Infect. Dis.*, 1939, **65**, 306.

4 Bryan, W. R., Beard, D., and Beard, J. W., *J. National Cancer Institute*, in press.

5 Bryan, W. R., and Beard, J. W., *J. Infect. Dis.*, in press.

† We are greatly indebted to Dr. L. G. Longsworth, Rockefeller Institute for Medical Research, for his generous advice in the construction and operation of the instrument employed in these studies.

6 Tiselius, A., *Trans. Faraday Soc.*, 1937, **33**, 524.

in boundary shape were recorded photographically by the refractive index method of Svensson.[7]

The pH regions available for study of the intact protein are determined[8] by the limits of molecular stability in both acid and alkaline ranges and by insolubility near the isoelectric point. An example of the results obtained in the acid range of solubility is shown in Fig. 1 in the series of curves photographed after successive time intervals over a period of 5.5 hours at pH 3.78. The abscissas represent distances in the direction of migration of the protein boundary into protein solution while the ordinates are proportional to the refractive index gradient or protein concentration gradient in the

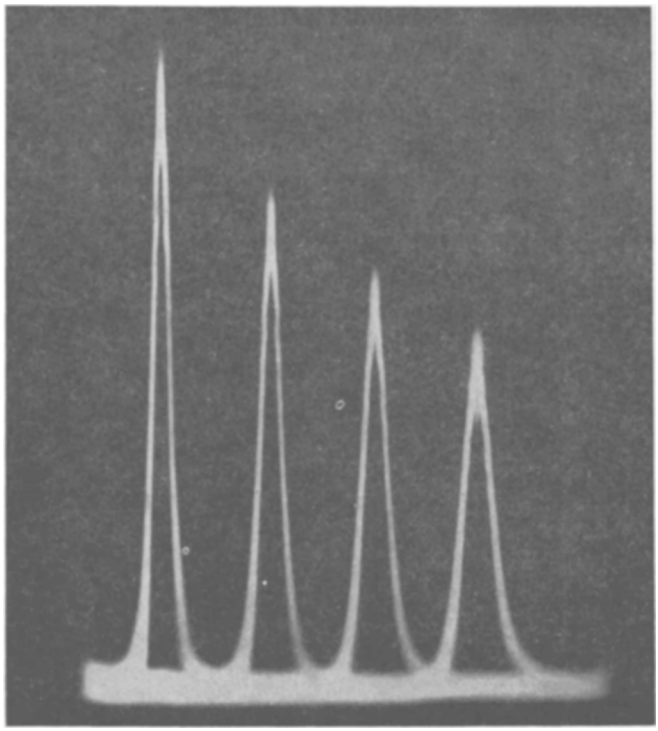

FIG. 1.

Svensson diagrams made with the rabbit papilloma virus protein. The initial peak was photographed at the beginning of the experiment; the succeeding peaks were photographed at intervals of 110 minutes for 5½ hours. The electric field was 3.26 volts/cm; the ionic strength, 0.1; and the pH, 3.78. The photographs record movement of the boundary (from left to right) into protein. The mobility was $+3.70 \times 10^{-5}$ cm²/sec./volt.

[7] Svensson, H., *Kolloid Z.*, 1939, **87**, 181.

[8] Beard, J. W., and Wyckoff, R. W. G., *J. Biol. Chem.*, 1938, **123**, 461.

boundary at that point. Throughout the experiment the boundary remained single and sharp, exhibiting no evidence of splitting. With continuous migration, slight spreading of the boundary occurred due to combined influence of diffusion and inhomogeneity of particle charge. At 0°C diffusion of the large molecules is very small and most of the spread is attributable to the latter factor. The extreme sharpness of the last single peak of Fig. 1 shows a remarkably high degree of uniformity of particle charge, indicative of corresponding homogeneity of the papilloma-virus protein in the acid range below the isoelectric point. Preliminary studies have been made in the region of solubility above the isoelectric point. Here, the protein moved with a single sharp boundary with slightly more spread and skewness in the final picture than in that of Fig. 1. In none of the studies thus far made (between pH 3.78 and pH 7.7) has there been any evidence of boundary splitting.

The results show that the papilloma protein is extremely well suited to studies in the Tiselius apparatus. Under the conditions described solutions of this animal virus protein exhibit on both sides of the isoelectric point a high degree of electrophoretic homogeneity comparable to or possibly greater than that of the most homogeneous hemocyanins[9] and crystalline proteins.[10] These findings combined with those of sedimentation studies provide a valuable criterion of the physical state of the material since the degree of homogeneity observed with the papilloma protein has been seen thus far only with solutions of a single molecular species.

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Additional Observations on Vitamin K-Deficient Diets.

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In recent papers Almquist[1, 2] emphasizes the precautions necessary in the care and housing of test animals in order to prevent bacterial

[9] Tiselius, A., and Horsfall, F., Jr., *Arch. f. Kemi, Mineralogi och Geologi*, 1939, **13** A, 1.

[10] Longsworth, L. G., Cannan, R. K., and MacInnes, D. A., *J. Am. Chem. Soc.*, 1940, **62**, 2580.

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