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Qualitative Separation of Proteins of Rat Serum by Electrophoresis.

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The electrophoretic migration of the proteins in rat serum was studied in the apparatus modified by Tiselius from older designs.[1] Toeppler's "Schlieren" method was used for following the movement of the differently charged fractions.

Three different fractions, one albumin and 2 globulins, separate from normal rat serum under the influence of a low difference of potential (about 135 volts, giving about 26 milliamperes current). Electrophoresis was continued as long as 11 hours without the occurrence of any further split in the protein bands. Human and horse sera,[2] studied in this and other laboratories, differ from rat serum in that they contain an albumin and 3 globulin fractions. (Fig. 1.)

Rat serum continued to yield the same number of fractions at different pH values extending on both sides of the isoelectric points of the proteins from pH 4.0 to 7.5. (Fig. 2.) An exception occurs when a globulin is partially precipitated at a pH near its isoelectric point. In this case a fourth fraction appears.

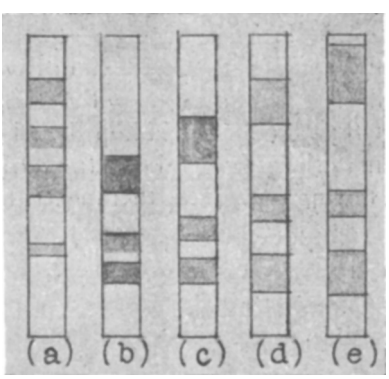

FIG. 1.

Electrophoresis of horse serum (a), and rat serum at pH 6.23 3, 4, 5, and 6 hours after potential was applied (b, c, d, and e).*

1 Tiselius, A., *Tr. Farad Soc.*, 1937, **33**, 524.

2 Tiselius, A., *Särtryck ur Svensk Kemisk Tidskrift*, 1938, **50**, 66.

* Buffer solutions are .02 molar with respect to the phosphate ovine acetate ion and .15 molar with respect to NaCl.

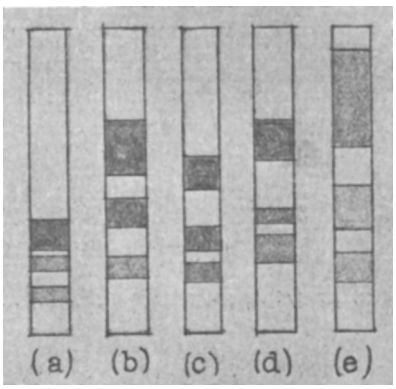

FIG. 2.
Electrophoresis of rat serum at pH values of 4.0 (a), 4.95 (b), 6.23 (c), 7.0 (d), and 7.5 (e).*

If electrophoresis is carried out at the low potential until the 3 fractions have been well separated and the potential is then raised to 250 or 300 volts (50 to 60 milliamperes current) a disintegration of the 2 globulins takes place. At pH 7.5, the α globulin gives rise to a fraction travelling with greater speed and appearing, consequently, between the albumin and the α globulin. The β globulin yields a fraction moving with less speed, and visible as a rather sharp line following this fraction. At a pH value below the isoelectric point of the globulins, 4.95, the fraction separating from the β globulin precedes it towards the negative electrode; the fraction with its source in the α globulin precedes the albumin towards the positive electrode. These new fractions persist after the voltage is returned to its original value. (Fig. 3.)

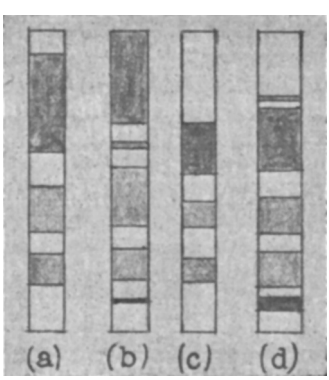

FIG. 3.
Electrophoresis of rat serum, showing serum at pH 7.5 (a) 135 volts, (b) 270. At pH 4.95 (c) 135 volts, (d) 270 volts.*

These experiments appear to confirm the view, now held by several investigators besides ourselves,[3] that globulins may occur in complexes, easily resolved into their components. In this instance the resolution has been effected by applying a sufficient potential gradient.

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Assay of Vitamin K Concentrates.*

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Since our extensive experience in the assay of estrogens has led to an appreciation of the importance of the contribution of Trevan[1] to bioassay, our work on the assay of Vitamin K was directed toward the establishment of standard curves of response to the administration of the vitamin. Based on Trevan's principles a curative method of assay which appears to have certain advantages over methods previously used by Schønheyder,[2] Almquist and Stokstad,[3] and Almquist, Mecchi and Klose[4] has been developed.

Experimental Diet. In our earlier work, difficulty in consistently producing the deficiency was encountered. It seems that this was due chiefly to diets that were not entirely devoid of Vitamin K. The diet now used is one described by Almquist.[5] Its composition is: fish meal, 17.5 parts; dried brewer's yeast, 7.5 parts; ground polished rice, 73 parts; sodium chloride plus small amounts of cupric and ferrous sulfates, 1.0 part; and cod liver oil 1.0 part. The fish meal and yeast were extracted with hot isopropyl ether before incorporation in the diet.

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3 Jameson, E., *Symposia on Quantitative Biology*, Cold Spring Harbor Biological Lab., 1938, **6**, 331; Sørensen, S. P. L., *Kolloid Z.*, 1930, **53**, 102; Kendall, F. E., *J. Clinical Invest.*, 1937, **16**, 921; McFarlane, A. S., *Biochemical J.*, 1935, **29**, 660, etc.; and others.

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1 Trevan, J. W., *Proc. Roy. Soc.*, 1927, **101**, 483.

2 Schønheyder, F., *Biochem. J.*, 1936, **30**, 890.

3 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **14**, 235.

4 Almquist, H. J., Mecchi, E., and Klose, A. A., *Biochem. J.*, 1938, **32**, 1897.

5 Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.