[CONTRIBUTION FROM THE DEPARTMENTS OF PHYSICAL CHEMISTRY AND MEDICINE, HARVARD MEDICAL SCHOOL, AND THE MEDICAL CLINIC OF THE PETER BENT BRIGHAM HOSPITAL]

# Serum Albumin. II. Identification of More than One Albumin in Horse and Human Serum by Electrophoretic Mobility in Acid Solution

BY JOHN A. LUETSCHER, JR.

The albumins of serum have constituted a problem of importance both for protein chemistry and clinical medicine. The serum albumins of the horse are readily crystallizable, and those of man have been crystallized, though with greater difficulty.[1,2] The beautifully crystalline albumin of normal horse serum has, however, been shown not to consist of a single chemical individual, as judged by solubility,[3] although it appears to be homogeneous as to molecular weight as judged by the ultracentrifuge,[4] and to migrate with uniform velocity in the electric field at neutral reaction.[5]

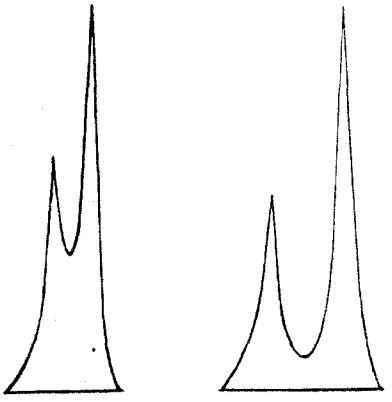


Fig. 1.—Schlieren diagrams (diaphragm level against distance moved) of electrophoresis at *p*H 4.0 and ionic strength 0.02 of (a) horse serum albumin, and (b) normal human serum albumin. Area under each peak is proportional to concentration of that component.

In the preceding paper,[6] McMeekin has succeeded in purifying crystalline serum albumin so as to yield at least one component of constant solubility. This was accomplished by "crystallization of horse serum albumin from salt-free solution" following an observation of J. D. Ferry.[7] The crystallization as an albumin sulfate was carried out at *p*H 4.0 and 25°. The serum albumin so fractionated previously had been crystallized repeatedly so as to free it from carbohydrate and had a single electrophoretic mobility in the Tiselius apparatus at neutral reactions where it has been studied as a function of *p*H, ionic strength, and protein concentration.

It seemed important to investigate the electrophoretic mobility of horse serum albumin at the *p*H at which Ferry and McMeekin have demonstrated that a portion of the albumin can be crystallized as the sulfate. Measurements were therefore undertaken on the same carbohydrate-free crystalline albumin that served as starting material for McMeekin's investigations[6] and which migrated in the electric field with uniform mobility at a great variety of ionic strengths at neutral reactions. When studied in acetate buffers at *p*H 4.0 of ionic strength 0.02, however, two boundaries were clearly visible, as represented in Fig. 1a. Migration was followed by the Toepler–Schlieren method,[8] ordinates and abscissas representing diaphragm level and distance moved. The amount of protein is proportional to the area under the curves. The albumin mobilities were 6.80 and 6.44 $\times 10^{-5}$ cm.$^2$/volt-sec., respectively, about 60% of the protein moving at the faster, 40% at the slower, rate. The protein in this solution, which thus revealed two boundaries when studied at *p*H 4.0, subsequently migrated with a single boundary at *p*H 7.4 of the same mobility as before.

When normal human serum albumin was investigated under precisely the same conditions, two boundaries were also clearly distinguishable, as represented in Fig. 1b. The mobilities of the two human serum albumin components at *p*H 4.0 and $\Gamma/2$ 0.02 were 7.25 and 5.95 $\times 10^{-5}$ cm.$^2$/volt-sec., respectively. The component of greater mobility of human serum albumin therefore moves appreciably faster as a cation than the faster component of horse serum albumin, and the slower component of human serum albumin more slowly than the slower component of horse serum albumin under these conditions. The

(1) Adair and Taylor, *Nature*, **135**, 307 (1935).
(2) Kendall, personal communication.
(3) Sörensen, *Compt. rend. trav. Lab. Carlsberg*, **18**, No. 5 (1930).
(4) Svedberg and Sjogren, THIS JOURNAL, **50**, 3318 (1928).
(5) Tiselius, *Biochem. J.*, **31**, 1464 (1937).
(6) McMeekin, THIS JOURNAL, **61**, 2884 (1939).
(7) Reported by Ferry to the Division of Biological Chemistry at the 96th meeting of the American Chemical Society, Milwaukee, September 7, 1938.
(8) An automatic camera for producing these diagrams has been described by Longsworth, THIS JOURNAL, **61**, 529 (1939).

separation achieved between the components of human serum albumin during electrophoresis is thus by far the greater, as is indicated by the greater separation of the peaks in Fig. 1b than in 1a. The protein migrating with the greater mobility represented approximately 67% and that with the slower mobility 33% of the albumin. Although there is approximately the same proportion of components in horse and man, the difference between the mobilities is sufficient to render practicable electrophoretic separation under these or closely similar conditions, at least in the case of the human protein. Human serum albumin, as in the case of the horse, migrates uniformly at neutral reactions.

Although the loss of serum proteins into the urine in certain diseases involves not only the albumin, but also the globulin fractions, the albumin molecules are generally lost in larger amount. It seemed desirable to investigate the albumin components of blood and urine from patients with certain types of kidney and liver disease. In the nephrotic syndrome, where large amounts of serum albumin are known to be lost and where the concentration of globulins (especially the $\alpha$- and $\beta$-fractions of Tiselius) and fibrinogen in blood is increased, the albumin both in the blood and the urine has been investigated under the same conditions as that of normal human serum albumin, namely, in acetate buffers at $p$H 4.0, and found to be divisible into two components. Unlike normal human albumin, however, the component of slower mobility has been found to be present in larger amount (Fig. 2b). Since the ratio of components appears to be the same in both urine and blood, this abnormality cannot be considered as due to urinary excretion but rather to a difference in protein production. The total amount of albumin is much reduced in these cases, as is indicated by comparing the shaded area under the curves, representing the albumins of nephrotic syndrome, with the area under the outline curve of the normal serum. Taking average concentrations of the normal components, respectively, as 1.3 and 2.7 g. per hundred cc., the concentration of the slower component would be reduced to one-sixth but of the faster component almost to one-thirtieth that of the normal. It follows that the production of the slower component is less affected than that of the faster. It was the urinary albumin from a similar case that had "a dielectric constant increment of 0.5 per gram per liter, higher than any thus far obtained for crystallized horse serum albumin, and a mean relaxation time in dilute solution of $0.2 \times 10^{-6}$ seconds,"[9] close to that observed for horse serum albumin.[10]

The above condition may be contrasted with that of the blood and urine of patients in the non-edematous end-stage of nephritis. Then the blood proteins approximate the normal picture, both as to the albumins and the globulins, although there would appear in the cases thus far investigated to be very little loss of the slower and a somewhat greater loss of the faster moving component (Fig. 2a).

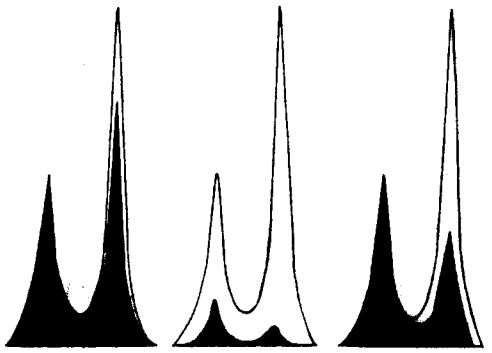


Fig. 2.—Schlieren diagrams of electrophoresis of pathological human albumins compared in each case with the normal picture. Concentrations of components in normal serum are 1.3 and 2.7 g. %; in terminal nephritis (a), 1.2 and 1.9; in nephrotic syndrome (b), 0.24 and 0.10; and in cirrhosis (c), 1.3 and 0.9 g. %, respectively. Ratio of albumins in urine was same as in serum.

In cirrhosis with ascites, where the principal factor in the lowering of plasma proteins is probably a failure of production, the albumin fraction may be much lowered, and in such cases the ratio of albumin components at $p$H 4.0 resembles that of the nephrotic syndrome, with the slower component predominating (Fig. 2c). Indeed, in cases thus far investigated no, or very slight, diminution in the concentration of the slower moving albumin has been detected, whereas the fast moving component has been reduced to one-third of the normal level. The role of the albumins which readily can be distinguished by electrophoresis at $p$H 4.0 is clearly different. That the difference in composition is not due to a gradual change in part of the albumin under

(9) Friend, Ferry and Oncley, *J. Biol. Chem.*, **123**, Proc. XXXIX (1938).

(10) Ferry and Oncley, THIS JOURNAL, **60**, 1123 (1938).

these conditions has been demonstrated by studying electrophoretic mobility of human serum albumin soon after equilibration with acetate buffer and again after a period of some days at 4° when no alteration in the electrophoretic mobility of either component or in the ratio of the components was detected.[11]

The separation and characterization not only of the albumin but also of the various globulin components of normal and pathological sera[12] are being investigated further.

## Summary

1. Although crystalline serum albumin has been demonstrated to consist of more than one chemical individual, it has been reported to migrate with uniform velocity both during ultracentrifugation and electrophoresis at neutral reactions.

2. At *p*H 4.0, where a fraction of horse serum albumin crystallizes as a sulfate, the protein exhibits two boundaries in the Tiselius electrophoresis apparatus, which migrate, respectively, with mobilities of 6.80 and 6.44 × $10^{-5}$ cm.²/volt-sec. in acetate buffers of *p*H 4.0 and Γ/2 0.02.

3. Human serum albumin also consists of at least two components, migrating with velocities of 7.25 and 5.95 × $10^{-5}$ cm.²/volt-sec., respectively, under the above conditions.

4. In normal sera, the faster moving component constitutes nearly two-thirds of the albumin, but in certain pathological conditions in man, both serum and urinary albumins show a greater diminution of the faster component, leaving the slower component preponderant.

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(11) Several of the pathological specimens were made available through the kindness of Dr. Allan M. Butler of the Children's Hospital, Boston, Mass.

(12) Studies of this kind have been reported by Stenhagen, *Biochem. J.*, **32**, 714 (1938); Blix, *Z. exptl. Med.*, **105**, 595 (1939); MacInnes and Longsworth, *Science*, **89**, 438 (1939).

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[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

# The Synthesis of Certain Substituted Quinolines and 5,6-Benzoquinolines

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The synthesis of ergoline (I) and of several of its mono- and dimethyl derivatives by the sodium reduction of 3′-amino-5,6-benzoquinoline-7-carboxylic acid lactam and of the corresponding methyl derivatives already has been reported.[1] Although the yields were only about 5 to 10%, the method appeared to be of fairly general application to the alkyl derivatives. When, however, a free or esterified carboxyl group was present in the pyridine ring, the isolation of a crystalline ergoline derivative became extremely difficult. The preparation of ergoline-7-carboxylic acid in a yield of about 1% and in still somewhat impure condition already has been reported,[2] but all attempts to prepare the isomeric ergoline-8-carboxylic acid have given only amorphous products. This plan of attack on the problem of the synthesis of dihydrolysergic acid has therefore not been so promising as had been anticipated.

In the course of this work several new methods of synthesis of certain quinoline and benzoquinoline derivatives were developed, which are the subject of the present paper.

Conrad and Limpach[3] first prepared 4-hydroxyquinaldine (II) by condensation of aniline with acetoacetic ester at room temperature followed by cyclization at 250°, and Limpach[4] forty-four years later improved the yield from about 30 to 90–95% by the use of mineral oil as a diluent in the cyclization step.

This method of synthesis has now been found to be sufficiently general to be applicable not only to substituted anilines and β-naphthylamines, but also has been extended to the synthesis of 4-hydroxyquinoline-3-carboxylic acids (III) by the use of ethoxymethylenemalonic ester and of 4-hydroxyquinaldine-3-carboxylic acids (IV) by the use of acetylmalonic ester.

As examples, aniline condensed with ethoxymethylenemalonic ester to give anilidomethylenemalonic ester, as reported by Claisen.[5] Cyclization of the latter in mineral oil gave the previously known[6] 4-hydroxyquinoline-3-carboxylic acid (III).

$$C_6H_5NH_2 + C_2H_5OCH{=}C(COOR)_2 \longrightarrow C_6H_5NHCH{=}C(COOR)_2$$

(1) W. A. Jacobs and R. G. Gould, *J. Biol. Chem.*, **120**, 141 (1937).

(2) W. A. Jacobs and R. G. Gould, *ibid.*, **126**, 67 (1938).

(3) M. Conrad and L. Limpach, *Ber.*, **20**, 944 (1887).

(4) L. Limpach, *ibid.*, **64**, 969 (1931).

(5) L. Claisen, *Ann.*, **297**, 77 (1897).

(6) R. Camps, *Ber.*, **34**, 2714 (1901).