the table show clearly that the ratio of fast to slow forms increases regularly with decreasing *p*H. In fact, quantitative analysis of the composition *vs.* *p*H data indicate almost perfect agreement with an equilibrium which is third order in hydrogen ion concentration. This same *p*H dependence was found previously for the optical rotation, though the inflection occurred almost one *p*H unit lower, a result which may be due to the difference in ionic strength or temperature.[3]

TABLE I

SUMMARY OF RESULTS

*p*H	Percentage composition Slow	Fast	Mobilities, cm.²/volt·sec. × 10⁵ Slow		Fast
3.42	0	100			+9.7
3.62	19	81	+7.8		+8.6
3.75	23	77	+6.6		+7.4
3.82	23	77	+5.2		+6.6
3.90	27	73	+4.5		+5.7
4.00	60	40	+3.5		+5.3
4.11	64	36	+2.5		+4.1
4.16	67	33	+2.3		+3.7
4.30	86	14	+1.2		+2.6
4.55	94	6	−1.0	−2.6	+0.8

Electrophoretic resolution indicates the equilibrium reaction to be relatively slow. However, variation of the dialysis time from 19 to 75 hours yielded no trends in composition. The half-life thus cannot exceed a few hours at the temperature of electrophoresis and dialysis (0°). (Very similar, though less complete, results have been obtained at 0° in 0.1 molar chloride, and in 0.02 molar chloride at 25°.)

Electrophoretic analyses were conducted in HCl–NaCl systems of constant ionic strength 0.02. The protein concentration employed was only 0.2% under which conditions the ascending and descending boundaries were reasonably enantiographic, two boundaries of roughly similar area and mobility appearing in both limbs. Some experiments were conducted at 0.05% protein yielding the same qualitative results; however, lack of sensitivity in the Schlieren optical system prohibited quantitative analysis of the composition.[9] At concentrations much above 0.2% enantiography is lost.

The protein preparation employed[10] exhibited relatively little electrophoretic inhomogeneity above *p*H 4.6, being the best in this regard of some half-dozen samples tested. At *p*H 4.6, the main peak showed definite evidence of a split into two components, in agreement with previous findings of three components in the isoelectric region.[7] As noted in the table the "slow" and "fast" forms actually have opposite sign of charge at this *p*H.

The observations reported here should open the way to a much more detailed elucidation of the peculiar low *p*H behavior of this protein. Results of such studies, together with a more detailed exposition of the results outlined here, will appear in due course.

(9) All electrophoretic analyses were performed in a Perkin–Elmer instrument equipped with scanning camera.

(10) Pentex, Lot A 1201. Ultracentrifugal analysis indicated the presence of a few per cent. of a faster sedimenting component, presumably aggregated material.

We are indebted to the National Cancer Institute, National Institutes of Health, for a grant in support of this research, and to the National Science Foundation for funds which provided equipment used.

DEPARTMENT OF CHEMISTRY
PURDUE UNIVERSITY — KOICHIRO AOKI
LAFAYETTE, INDIANA — JOSEPH F. FOSTER

RECEIVED APRIL 20, 1956

THE EFFECT OF ION BINDING ON PROTEIN STRUCTURE. I. INFLUENCE OF ACETATE ON THE ELECTROPHORETIC BEHAVIOR OF SERUM ALBUMIN[1]

Sir:

We wish to describe a new effect of acetate buffer on the structure of proteins. This effect is illustrated by experiments which show that the electrophoretic behavior of bovine serum albumin (BSA) depends upon the concentration of acetate buffer (NaAc–HAc) in the supporting medium. Representative electrophoretic patterns are shown in Figs. 1 and 2. Although these patterns are complex, it is clear that substitution of NaAc–HAc for NaCl in the supporting medium results in an increase in the area under the faster moving bound-

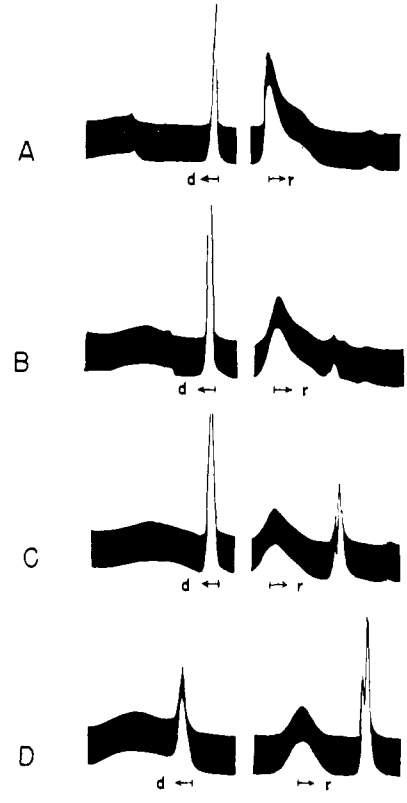

Fig. 1.—Electrophoretic patterns of Armour bovine serum albumin in various buffer solvents at *p*H 4.7: A, 0.002 ionic strength NaAc–HAc + 0.023 ionic strength NaCl; B, 0.005 NaAc–HAc + 0.020 NaCl; C, 0.010 NaAc–HAc + 0.015 NaCl; D, 0.025 NaAc–HAc. Protein migrated toward the cathode. The product of field strength and time is approximately the same in all these experiments.

(1) Supported in part by a research grant from the National Institutes of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service; and in part by the Damon Runyon Fund and the American Cancer Society.

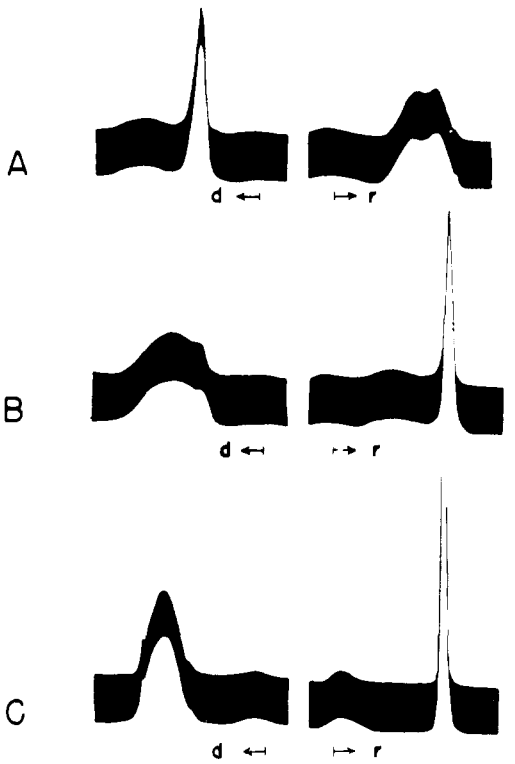

Fig. 2.—Electrophoretic patterns of Armour bovine serum albumin in various buffer solvents at *p*H 4.0: A, 0.002 ionic strength NaAc–HAc + 0.198 ionic strength NaCl; B, 0.005 NaAc–HAc + 0.195 NaCl; C, 0.20 NaAc–HAc. Protein migrated toward the cathode. The product of field strength and time is approximately the same in all these experiments.

aries, *i.e.*, those corresponding to more positively charged components, at the expense of slower moving ones. That this represents an effect of increasing Ac^- and/or HAc rather than decreasing Cl^- is indicated by the observation that the patterns obtained in 0.01 ionic strength NaAc–HAc, *p*H 4.7, are essentially the same as those obtained in 0.01 NaAc–HAc + 0.015 NaCl (the chief difference being that the more rapidly moving boundary in the rising pattern was not resolved into two peaks at the lower ionic strength).[2]

While binding of acetate has been either demonstrated or postulated in a number of protein systems, this is the first demonstration that such binding has a major effect on protein structure. (Such binding does not, however, change the sedimentation constant.) Recognition of this effect increases our understanding of the structure of BSA. The non-enantiographic nature of the electrophoretic patterns of this protein has been interpreted by Longsworth and Jacobsen[3] to indicate continual readjustment of an equilibrium across the moving boundaries. But other workers assume that BSA is a simple mixture of proteins.[4] A solution of BSA may well contain molecules which differ chemically.[5] However, we conclude from our experiments that BSA is indeed an equilibrium mixture of electrophoretic components; that any chemically different molecules participate in this equilibrium; and that the equilibrium composition depends upon the composition of the supporting medium, particularly the acetate concentration.[6] Two independent observations support these conclusions, first, if at the end of the usual moving-boundary electrophoresis experiment of duration *t*, the polarity of the electrodes is reversed and the experiment continued for another length of time *t*, then the final boundaries which have now returned to their initial positions are not single symmetrical ones as expected of a simple mixture of proteins but are complex showing as many as three distinct, although poorly resolved peaks. This suggests that an equilibrium was being continuously readjusted as the boundaries returned to their initial positions. Experiments of this type were carried out at several ionic strengths and concentrations of NaAc-HAc. The second piece of evidence is afforded by small differences in the ultraviolet absorption spectra of BSA in 0.1 NaAc–HAc and in 0.002 NaAc–HAc + 0.098 NaCl. These differences are confirmed by a differential spectrum which shows two main bands at 2350–2400 and 2900Å., and indicate that binding of Ac^- and/or HAc by BSA produces structural changes in the protein, perhaps due to making or breaking of hydrogen bonds.

The effect described above has also been observed with ovalbumin and γ-globulin and accounts for the complex electrophoretic patterns observed[7] for these proteins in certain buffers. Experiments are in progress to elucidate the nature of the effect of various buffer constituents (such as homologous series of aliphatic acids) on protein structure.

(2) The possibilities that the complex patterns arise from electrical anomalies or as a result of poor buffering action, have also been eliminated.

(3) L. G. Longsworth and C. F. Jacobsen, *J. Phys. Colloid Chem.*, **53**, 126 (1949).

(4) R. A. Alberty, *ibid.*, **53**, 114 (1949).

(5) H. A. Sober, F. J. Gutter, M. M. Wyckoff and E. A. Peterson, THIS JOURNAL, **78**, 756 (1956).

(6) The electrophoretic patterns of BSA are also dependent on whether the buffer cation is Na^+ or K^+, *p*H, and to some extent ionic strength.

(7) A. Saifer and H. Corey, *J. Biol. Chem.*, **217**, 23 (1955); Abstracts of Papers, 128th Meeting American Chemical Society, Minneapolis, Minn., Sept. 11–16, 1955, p. 40-C.

CONTRIBUTION NO. 43 FROM THE
DEPARTMENT OF BIOPHYSICS ROBERT A. PHELPS
FLORENCE R. SABIN LABORATORIES JOHN R. CANN
UNIVERSITY OF COLORADO MEDICAL CENTER
DENVER, COLORADO

RECEIVED APRIL 2, 1956

SYNTHESIS AND ANABOLIC ACTIVITY OF 4-SUBSTITUTED TESTOSTERONE ANALOGS

Sir:

We wish to make a preliminary announcement of the synthesis and anabolic activity of 4-substituted testosterone analogs.

Treatment of testosterone with alkaline hydrogen peroxide gave a mixture of the β- and α-epoxides, which could be separated by crystallization: 4β,5-oxidoetiocholane-17β-ol-3-one, m.p. 156–157°, $(\alpha)_D$ +145° and 4α,5-oxidoandrostane-17β-ol-3-one, m.p. 147–148°, $(\alpha)_D$ −33° [Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.86; H, 9.35 and C, 75.16; H, 9.28]; acetates m.p. 155–157°, $(\alpha)_D$ +130° ($CHCl_3$) and m.p. 172–173°, $(\alpha)_D$