

In order to prove that no decomposition of  $\alpha$ -chloropropionic acid and lactic acid took place during this resolution, amide derivatives of the recovered acid fractions were prepared according to standard methods.<sup>11</sup> The melting points of these derivatives indicated that no decomposition had occurred.

### Discussion

The mechanism of the above reaction is not completely understood but it is probably more complex than has been indicated here. This is shown, for example, by the fact that when carbonato-bis-*levo*-propylenediamine cobalt chloride is evaporated with an excess of tartaric acid and then taken back into solution, the excess tartrate is not completely precipitated by the addition of the calculated quantity of barium hydroxide. Moreover, it is difficult to understand why the resolution of  $\alpha$ -chloropropionic acid and lactic acid both proceeded to the extent of approximately 30% whereas the resolution of tartaric acid was much less. These problems will be studied further.

The supposition that  $\alpha$ -chloropropionic acid acts primarily as a monodentate coordinating agent was supported by precipitation of the uncoordinated  $\alpha$ -chloropropionate fraction by silver ions at a pH of about 6. The result is only approximate, for the silver salt of the acid is fairly soluble and was found to decompose slowly into silver chloride and lactic acid on standing in solution. However, the results were sufficiently exact

(11) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," Third Ed., John Wiley and Sons, Inc., New York, N. Y., 1948, pp. 158, 222-223.

to indicate that one-half of the acid originally added was not readily precipitated by silver ion, so that two acid anions must have entered each coordination sphere.

Since it appears that the dextro form of the acid enters the coordination sphere of the carbonato-bis-*levo*-propylenediamine cobalt(III) complex preferentially, this reaction could be used as a method of determining the configuration of any optically active organic acid which would coordinate either as a monodentate or a bidentate group. If the molecule or anion readily entered the coordination sphere of the complex it should have a dextro configuration regardless of the sign of rotation. Comparison of this result with a resolution of a racemic mixture of the acid by this method should yield a clear indication of steric configuration.

It should be mentioned that this procedure has also been extended to the resolution of *rac*- $\alpha$ -bromopropionic acid, though the results in this case are purely qualitative. The resolution proceeds in exactly the same manner, with the levorotatory isomer coordinating preferentially.

Though a resolution of 30% is only partial it is significant. The resolution of *rac*-lactic acid, especially, proceeds much more readily by this method than by the usual methods of recrystallization of alkaloid salts of the acid. The procedure as described also appears to be of a general nature and should be adaptable to the resolution of many other organic acids.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY<sup>1</sup>]

## Acid-Base Titration, Viscosity and Density of $\alpha$ -, $\beta$ - and $\gamma$ -Casein

BY N. J. HIPPI, M. L. GROVES AND T. L. McMEEKIN

RECEIVED MAY 17, 1952

Titration curves, viscosities and densities of  $\alpha$ -,  $\beta$ - and  $\gamma$ -casein show that these proteins differ and that the variation in properties is related to their amino acid compositions. Results obtained on preparations of these proteins separated by alcohol or urea were the same as those obtained by the milder procedure of isoelectric precipitation at 2°, indicating that no change in structure was produced by alcohol or urea.

Numerous studies have been reported on the acid- and base-combining capacity of casein,<sup>2-4</sup> its viscosity and its density.<sup>5-8</sup> The demonstration by Mellander<sup>9</sup> that casein is a mixture of at least three components, and the separation of these components in our laboratory make it possible for the first time to determine the properties of these pure caseins.

Since the components of casein are difficult to separate, it might be expected that they would have only small differences in properties. The present study showed, however, that their titration curves, viscosities and densities are markedly different.

### Materials and Methods

**Caseins.**— $\alpha$ -,  $\beta$ - and  $\gamma$ -casein were prepared by several methods, namely, isoelectric precipitation from water at 2°, differential solubility in 50% aqueous alcohol with change in pH and temperature, and differential solubility in various concentrations of aqueous urea. These methods have been described in detail by Warner<sup>10</sup> and Hipp, *et al.*<sup>11,12</sup> The unfractionated casein was prepared from unpasteurized bovine skim milk by acid precipitation, washed several times and purified by two isoelectric reprecipitations in the manner previously described.<sup>11</sup>

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) E. J. Cohn and Ruth E. L. Berggren, *J. Gen. Physiol.*, **7**, 45 (1924-1925).

(3) D. I. Hitchcock, *ibid.*, **16**, 357 (1932-1933).

(4) C. L. A. Schmidt, "The Chemistry of the Amino Acids and Proteins," Charles C. Thomas Publisher, Baltimore, Md., 1938, p. 744.

(5) H. Chick and C. J. Martin, *Z. Chem. Ind. Kolloide*, **11**, 102 (1912).

(6) M. A. Lauffer, *Chem. Revs.*, **31**, 561 (1942).

(7) C. L. Hankinson and D. R. Briggs, *J. Phys. Chem.*, **45**, 943 (1941).

(8) H. Chick and C. J. Martin, *Biochem. J.*, **7**, 92 (1913).

(9) O. Mellander, *Biochem. Z.*, **300**, 240 (1939).

(10) R. C. Warner, *THIS JOURNAL*, **66**, 1725 (1944).

(11) N. J. Hipp, M. L. Groves, J. H. Custer and T. L. McMeekin *ibid.*, **72**, 4928 (1950).

(12) N. J. Hipp, M. L. Groves, J. H. Custer and T. L. McMeekin *J. Dairy Sci.*, **35**, 272 (1952).

**Acid-Base Titration.**—Stock casein solutions were prepared by dissolving the air-dried protein with a minimum amount of 0.1 *N* sodium hydroxide. The concentration of the stock solution (about 2%) was determined by drying an aliquot to constant weight at 105° and correcting for sodium. This method gave the same value for protein concentration as was calculated on the basis of anhydrous weight of the protein used.

Reaction mixtures were prepared by addition of measured volumes of standard NaCl and HCl or NaOH to a measured volume (5 ml.) of stock solution, followed by dilution to a protein concentration of 1% and an electrolyte concentration of 0.05 ionic strength. To compensate for the variable addition of acid or base, the amount of NaCl added to the reaction mixture was adjusted to make the sum of acid or base and NaCl a constant. This method, described by Cannan, *et al.*,<sup>13</sup> is the simplest experimental approximation to a series of mixtures of constant ionic strength.

**pH Measurements.**—The pH of the reaction mixtures was determined by the Beckman Model G pH meter with the glass electrode at 25°. The special blue-glass high alkali electrode was used for the more alkaline solutions. The following buffer solutions with pH values at 25° determined by the National Bureau of Standards<sup>14</sup> were used to standardize the pH meter: (a) 0.05 *M* potassium hydrogen phthalate, (b) 0.025 *M* phosphate mixture, (c) 0.01 *M* sodium tetraborate, with pH values of 4.01, 6.86 and 9.18, respectively. Two additional solutions, 0.01 *M* hydrochloric acid plus 0.09 *M* potassium chloride, and 0.05 *M* potassium hydroxide were used, with pH values of 2.078 and 12.61, as given by Steinhardt and Harris.<sup>15</sup> When the pH meter was set at the proper pH with any of these buffers except the two most alkaline ones, each of the other buffers could be read to within 0.02 pH unit of the values assigned to them. When the high pH glass electrode was used, the same was true for the two most alkaline solutions. Reaction mixtures in the pH region of about 8.3 gave the same pH value with either glass electrode.

**Activity Coefficients.**—The observed pH value has been converted to the free hydrogen or hydroxyl ion by the relation  $\text{pH} = -\log [\text{H}^+] \gamma_{\text{H}^+} = \log [\text{OH}^-] (\gamma_{\text{OH}^-}/K_w)$ . In the calculation for the free hydrogen or hydroxyl ion in a solution containing protein, it is assumed that the activity coefficients depend only on the non-protein components of the system.<sup>16</sup> Apparent hydrogen ion activity coefficients in the absence of protein have been determined by measuring the pH of solutions of known molarities of hydrochloric acid at a total ionic strength of 0.05. These values are listed in Table I. Owing to inherent experimental difficulties at high pH values, calculation of the apparent hydroxyl ion activity coefficients from values obtained with the glass elec-

trode gives inconsistent results. The values were calculated, therefore, by using the activity coefficient values of Cohn and Berggren<sup>2</sup> and our determined hydrogen ion activity coefficients, as shown in Table I. The calculated hydroxyl ion activity coefficient values give a smooth curve, which fits the scattered determined values within experimental error (0.02 pH unit).

**Viscosity.**—Relative viscosities were measured with the Fenske modified Ostwald viscosimeter at 25.00 ± 0.02° in the usual manner. All stock solutions were prepared with 0.1 *N* NaOH and made to an ionic strength of 0.05 with sodium chloride and the concentration (grams per 100 ml.) determined by the method previously described. All the casein solutions for determination of viscosity had a pH within 6.4–6.9 except  $\gamma$ -casein, which had a pH of 7.5. The stock solutions were carefully filtered by pressure through a fine sintered-glass funnel, and dilutions were made with 0.05 *M* sodium chloride.

**Density in the Solid State.**—For several reasons the methods that have been used for determination of the dry density of powdered materials such as proteins are not entirely satisfactory. The method developed by Low and Richards,<sup>17</sup> which utilizes a density gradient and centrifugation is reliable. The samples for determination of dry density were first dried over P<sub>2</sub>O<sub>5</sub> under high vacuum at room temperature for 24 hours. They were then wet with a density gradient liquid having a density less than that of the solid, and subjected to a vacuum a number of times to remove occluded air. The density gradient tube was prepared by stroking the interface of two 5-cc. volumes of bromobenzene-xylene mixtures having a density difference of about 0.02 with a wire in the form of a flat spiral in a 16 × 150-mm. test-tube. A small amount of the suspended material of unknown density was introduced just below the surface of the density gradient liquid by means of a small glass tube. The gradient tube was then centrifuged for two min. at about 2400 r.p.m. and allowed to coast to a stop without any brake action. The choice of the density of the two liquids must be such that the protein is suspended in the middle third of the density gradient, since the upper and lower third do not always have a straight-line gradient. The dry density of the protein was determined by establishing the density of the liquid where the protein was suspended. This was done by introducing small drops of a salt (ZnCl<sub>2</sub>) solution of known density. The measurements reported here are accurate to ±0.001 in density units.

## Results and Discussion

**Acid-Base Titration.**—Figure 1 shows titration curves with HCl and NaOH of 1% solutions of  $\alpha$ -,  $\beta$ - and  $\gamma$ -casein and an equal mixture of  $\alpha$ - and  $\beta$ -casein at 0.05 ionic strength at 25°. The titration curve for each of the components of casein was independent of the method by which the components were prepared.

No changes in the pH of the reaction mixtures of the components of casein were detectable within the time interval of one min. to 72 hours after mixing over the entire pH range studied even in the insoluble region. The marked difference in the titration curves of  $\alpha$ - and  $\beta$ -casein (Fig. 1) is consistent with the analytical data for ionic groups reported by Gordon, *et al.*<sup>18</sup> The titration curves at high and low pH values permit an estimation of the maximum base- and acid-combining capacity of the casein components. Table II shows these values. The difference in the base-combining capacity of the three caseins is also evident and parallels the values obtained by anionic group analysis for  $\alpha$ - and  $\beta$ -casein. The values given for both  $\alpha$ - and  $\beta$ -casein, however (Table II), are 22 mols greater than the analytical values for anionic

TABLE I

APPARENT HYDROGEN AND HYDROXYL ION ACTIVITY COEFFICIENTS

Molarity hydrogen or hydroxyl ions	Cohn's value at given molarity and ionic strength		Constant ionic strength 0.05	
	1 <sup>a</sup> $\gamma_{\text{H}^+}$	2 <sup>a</sup> $\gamma_{\text{OH}^-}$	3 <sup>b</sup> $\gamma_{\text{H}^+}$	4 Calculated (1-3) $\gamma_{\text{OH}^-}$
0.008	0.930	0.930	0.910	0.910
.01	.92	.92	.890	.890
.015	.908	.902	.858	.852
.02	.90	.89	.831	.821
.03	.891	.873	.787	.769
.0368	.886	.865	.766	.745
.0390	.883	.862	.761	.740

<sup>a</sup> Interpolated from the values of Cohn and Berggren.

<sup>b</sup> Determined in the absence of protein by measuring the pH of solutions of known molarities of hydrochloric acid at a total ionic strength of 0.05.

(13) R. K. Cannan, A. H. Palmer and A. C. Kibrick, *J. Biol. Chem.*, **142**, 803 (1942).

(14) National Bureau of Standards Letter Circular LC 993.

(15) J. Steinhardt and M. Harris, *Natl. Bur. Standards J. Research*, **24**, 335 (1940) (RP 1286).

(16) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 454.

(17) B. W. Low and F. M. Richards, *THIS JOURNAL*, **74**, 1660 (1952).

(18) W. G. Gordon, W. F. Semmett, R. S. Cable and M. Morris, *ibid.*, **71**, 3293 (1949).

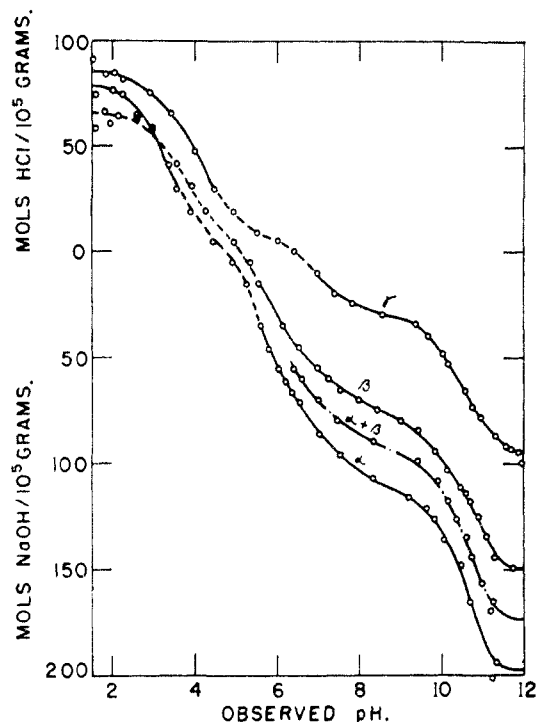


Fig. 1.—Acid-base titration curves for  $\alpha$ -,  $\beta$ - and  $\gamma$ -casein and an equal mixture of  $\alpha$ - and  $\beta$ -casein at protein concentration of 1%, 0.05 ionic strength and 25°. Ordinates are mols bound per  $10^5$  g. The dotted line is the pH region where, under the conditions of the experiment, all the protein was not soluble. For clarity, only a portion of the curve for the mixture of  $\alpha$ - and  $\beta$ -casein is given.

groups, including phosphoric acid, reported by Gordon,<sup>18</sup> namely, 176 and 128 equivalents per  $10^5$  g. for  $\alpha$ - and  $\beta$ -casein, respectively. Our calculated value for the base-combining capacity of unfractionated casein, based on a composition of 16 parts  $\alpha$ -, 4 parts  $\beta$ -, and 1 part  $\gamma$ -casein, is in agreement with the value of 183 mols per  $10^5$  g. reported by Cohn and Berggren<sup>2</sup> for several samples of casein. They also discussed the discrepancy between the greater base-combining capacity and the results of amino acid analysis.

TABLE II

MAXIMUM COMBINING CAPACITY OF $\alpha$ -, $\beta$ - AND $\gamma$ -CASEIN			
	$\alpha$	$\beta$	$\gamma$
Mols acid/ $10^5$ g. casein	78	66	85
Mols base/ $10^5$ g. casein	198	150	96
Grams casein/equivalent of acid	1282	1500	1176
Grams casein/equivalent of base	505	666	1042

The calculated maximum acid-combining capacity of unfractionated casein based on the combining capacity of its components is consistent with the value of 80 reported by Hitchcock<sup>3</sup> and the values of 60–90 given by Schmidt.<sup>4</sup>

**Stoichiometry.**—Considerable information can be obtained on the stoichiometry directly from the titration curve. Breaks in the titration curve or the transition points that are the result of differences in the ionization values of the ionizing groups are somewhat dependent on the ionic strength and do not always occur at the same pH for various

proteins.<sup>13,19,20</sup> In addition to the usual ionizing groups found in most proteins, the casein components contain amounts of phosphoric acid that must be considered in an analysis for anionic groups. According to Cannan, *et al.*,<sup>19</sup> a phosphate group in egg albumin may be expected to contribute two equivalents to the titration curve—one in the carboxyl region at about pH 2 and one in the imidazole region at about pH 7. It is not easy to establish from these curves definite transition points representing the ionization of specific groups. pH values of 6.35 and 9.3 have been selected, since these values give results for  $\alpha$ -casein that agree with the analytical data of Gordon, *et al.*<sup>18</sup> They are also consistent with the values given by Scatchard<sup>21</sup> for the binding of hydrogen ion for the various types of groups in other proteins under similar conditions. Support for the hypothesis that these values represent the ionization of definite groups in casein is furnished by the fact that the same values, when applied to  $\beta$ -casein, give figures in excellent agreement with the analytical data, in spite of the considerable difference in the relative number of ionizing groups in  $\alpha$ - and  $\beta$ -casein. At pH 6.35, the titration curves should give the number of carboxyl groups plus one equivalent of phosphorus and at pH 9.3 the number of carboxyl plus 2 phosphorus equivalents plus imidazole. Based on these pH values, the calculated values for the ionizing groups in Table III are in excellent agreement with the analytical results for  $\alpha$ - and  $\beta$ -casein. No comparative analytical values for  $\gamma$ -casein are available. Different pH values for the transition points have been used for other proteins, namely, 5.75 and 8.0 for human serum albumin,<sup>22</sup> 8.25 for  $\beta$ -lactoglobulin,<sup>13</sup> and 9.0 for egg albumin<sup>19</sup> for calculating the respective ionizing groups. Table III gives results obtained with these pH values for comparison with the preferred values for casein.

TABLE III

COMPARISON OF THE IONIZING GROUPS OF  $\alpha$ -,  $\beta$ - AND  $\gamma$ -CASEIN AS DETERMINED FROM TITRATION CURVES AND AMINO ACID ANALYSES

Mols H <sup>+</sup> dissociated	Mols per $10^5$ g.		
	$\alpha$	$\beta$	$\gamma$
At pH 6.35 (COOH + 1 equiv. of phosphate)	144	108	84
At pH 9.3 (COOH + 2 equiv. of phosphate + imidazole)	195	148	119
Anal. <sup>a</sup> COOH + 1 equiv. of phosphate	144	108	...
Anal. <sup>a</sup> COOH + 2 equiv. of phosphate + imidazole	195	148	...
At pH 5.75 <sup>b</sup>	120	88	78
At pH 8.0 <sup>c</sup>	181	136	111
At pH 8.25 <sup>d</sup>	184	139	112
At pH 9.0 <sup>e</sup>	190	145	116

<sup>a</sup> Gordon, *et al.*<sup>18</sup> <sup>b</sup> Average pH at which Tanford<sup>20</sup> obtained COOH for human serum albumin. <sup>c</sup> pH at which Tanford<sup>20</sup> obtained COOH + imidazole for human serum albumin. <sup>d</sup> pH at which Cannan<sup>13</sup> obtained COOH + imidazole for  $\beta$ -lactoglobulin. <sup>e</sup> pH at which Cannan<sup>19</sup> obtained COOH + imidazole + HPO<sub>4</sub> for egg albumin.

(19) R. K. Cannan, A. Kibrick and A. H. Palmer, *Ann. N. Y. Acad. Sci.*, **41**, 243 (1941).

(20) C. Tanford, *This Journal*, **72**, 441 (1950).

(21) G. Scatchard, *Amer. Scientist*, **40**, 76 (1952).

The titration curve for  $\alpha$ - and  $\beta$ -casein (Fig. 1) crosses at pH 3.0, indicating a significant difference in the number of charged groups having a  $pK$  of about 3.0. Examination of the analytical data does not reveal a satisfactory explanation on the basis of the normally accepted  $pK$  values of the groups involved. Uncertainties such as the actual site of the amide groups and the effect of the phosphoric acid on the  $pK$ 's of the groups combined in peptide linkage may be factors in explaining the crossing of the titration curves.

Warner<sup>10</sup> has shown that when  $\alpha$ - and  $\beta$ -casein are mixed the mobility of  $\alpha$ -casein decreases and the mobility of  $\beta$ -casein remains substantially the same. This indicates that the net charge of  $\alpha$ -casein decreases in the presence of  $\beta$ -casein and demonstrates an interaction of the two proteins. Since the titration curve is a measure of the charge on a protein, a reduction in charge due to interaction should be evident in a mixture of these proteins. The titration curve of an equal mixture of  $\alpha$ - and  $\beta$ -casein is exactly at the midpoint of the  $\alpha$ - and  $\beta$ -casein curves (Fig. 1). No interaction of these proteins could be demonstrated, therefore, by changes in net charge as deduced from the titration.

**Viscosity.**—The intrinsic viscosity  $[\eta]$  was determined by extrapolating the reduced viscosity,  $\eta_{sp}/C$ , to zero concentration. The results for  $\alpha$ -,  $\beta$ -,  $\gamma$ - and unfractionated casein, given in Fig. 2, are  $[\eta] = 0.105, 0.144, 0.095$  and  $0.125$  (100 ml./g.), respectively. The value of  $0.125$  is in good agreement with the value  $[\eta] = 0.13$  for unfractionated casein at pH 6.6 in the presence of  $0.01 M$  sodium chloride reported by Hankinson and Briggs.<sup>7</sup> The intrinsic viscosities of the casein components were independent of the method of preparation. Consequently only one plot is shown of each casein component (Fig. 2). The slope of the straight-line plots was independent also of the method of preparation except in one instance, that of  $\beta$ -casein prepared by the means of aqueous urea,<sup>12</sup> where some decrease in the slope was noted, though the intercept was the same as obtained for other preparations.

Data on protein viscosity have been used to interpret the asymmetry of the molecule. Simha<sup>22</sup> has developed a theoretical equation from the hydrodynamic behavior of ellipsoids, without any assumption of hydration, which relates a function of the axial ratio of the protein to the intrinsic viscosity. Mehl, Oncley and Simha<sup>23</sup> have calculated values of intrinsic viscosity of proteins by the Simha equation that correspond to various values of the axial ratio,  $b/a$ , for both rod- and disc-like particles. The values for axial ratios of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and unfractionated casein shown in Table IV were obtained by interpolation in the curves from the data of Mehl, *et al.*, which relate intrinsic viscosity to axial ratios. The apparent specific volumes of the casein previously reported<sup>24</sup> and the determined value of  $0.750$  for  $\gamma$ -casein were used for calculating intrinsic viscosities on a volume frac-

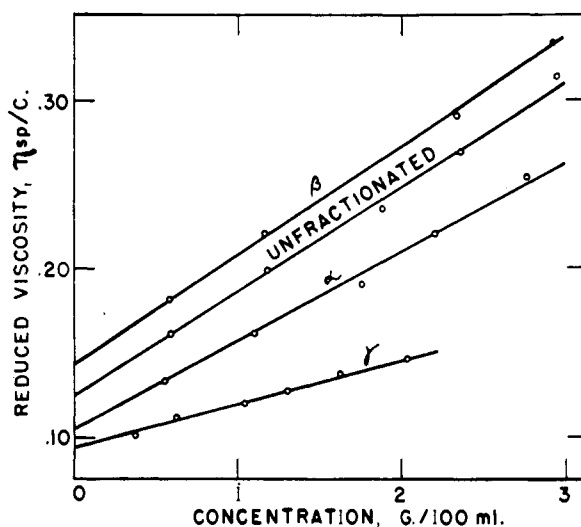


Fig. 2.—Viscosity of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and unfractionated casein at 0.05 ionic strength. The solutions had a pH within 6.4–6.9 except the  $\gamma$ -casein solution, which had pH 7.5.

tion basis. Values for the axial ratios of the caseins are somewhat higher than the values reported by Mehl for a number of proteins with the exception of gliadin and helix hemocyanin.

TABLE IV

AXIAL RATIOS OF  $\alpha$ -,  $\beta$ -,  $\gamma$ - AND UNFRACTIONATED CASEIN

Casein	$[\eta]_G^a$	Elongated $b/a$	Flattened
$\alpha$	14.0	10.2	18.8
$\beta$	20.5	13.2	28.6
$\gamma$	12.2	9.3	16.2
Unfractionated	17.6	12.0	24.0

<sup>a</sup>  $[\eta]$  volume fraction intrinsic viscosity.

Plomley, *et al.*,<sup>25</sup> reported a value of 9.6 for the axial ratio of unfractionated casein based on an assumed hydration of 0.4 g. of solvent per gram of protein, which is of the same order as our uncorrected value 12.0. The significance and value of the axial ratio in relation to the shape of the molecule as determined from viscosity has been discussed by Lauffer.<sup>6,26</sup> The observation that  $\beta$ -casein has a higher intrinsic viscosity than  $\alpha$ - or  $\gamma$ -casein was unexpected, since all previous measurements on these proteins except for the acid-combining capacity have shown that the properties of  $\beta$ -casein are intermediate between those of  $\alpha$ - and  $\gamma$ -casein. No independent measurements of the sizes and shapes of these caseins are available for comparison.

**Density of Caseins and Contraction of Volume on Dissolving.**—Chick and Martin,<sup>8</sup> who compared the density of casein and other proteins in the dry state and in solution, demonstrated that there is an apparent shrinkage of 5–8% in the volume of a protein when dissolved. It has been shown also that amino acids shrink in volume when dissolved, whereas simple uncharged organic molecules like amides change little in volume on dissolving, usu-

(22) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(23) J. W. Mehl, J. L. Oncley and R. Simha, *Science*, **92**, 132 (1940).

(24) T. L. McNeekin, M. L. Groves and N. J. Hipp, *THIS JOURNAL*, **71**, 3298 (1949).

(25) K. F. Plomley, H. G. Higgins and J. F. Hayes, *Nature*, **167**, 224 (1951).

(26) D. E. Green, "Currents in Biochemical Research," Interscience Publishers, New York, N. Y., 1946, chap. 16, p. 241.

TABLE V  
SPECIFIC VOLUMES OF PROTEINS IN THE SOLID STATE AND IN SOLUTION<sup>a</sup>

Protein	Solid state		Sp. vol. in soln., ml./g.	Volume change, ml./g.	No. of pairs of charged groups/10 <sup>5</sup> g. protein	Change in sp. vol. due to charged groups, ml./g.
	Density, g./ml.	Sp. vol., ml./g.				
Unfract. casein <sup>b</sup>	1.292	0.774	0.731 <sup>24</sup>	-0.044	110 <sup>18</sup>	0.022
α-Casein	1.293	.773	.728 <sup>24</sup>	- .045	115 <sup>18</sup>	.023
β-Casein	1.271	.787	.741 <sup>24</sup>	- .046	91 <sup>18</sup>	.018
γ-Casein	1.250	.800	.750	- .050	..	...
β-Lactoglobulin	1.247	.802	.751 <sup>c</sup>	- .051	115 <sup>18</sup>	.023
Egg albumin	1.269 <sup>8,d</sup>	.788	.744 <sup>e</sup>	- .044	91 <sup>18</sup>	.018
Serum albumin	1.275 <sup>8</sup>	.784	.734 <sup>f</sup>	- .050	100 <sup>20</sup>	.020

<sup>a</sup> Superscript numbers refer to footnotes in the text. <sup>b</sup> Artificial mixtures of α-, β- and γ-casein can be separated by centrifuging in an appropriate gradient density tube. However, no separation of the components of unfractionated casein could be obtained under similar conditions. <sup>c</sup> K. O. Pedersen, *Biochem. J.*, **30**, 961 (1936). <sup>d</sup> H. Neurath and H. B. Bull, *J. Biol. Chem.*, **115**, 519 (1936). <sup>e</sup> G. S. Adair and M. E. Adair, *Proc. Roy. Soc. (London)*, **120B**, 422 (1936). <sup>f</sup> J. F. Taylor, *Federation Proc.*, **9**, 237 (1950).

ally the volume in solution being slightly greater than that in the solid state.<sup>27</sup> These comparative values suggest that the contraction in volume on dissolving is associated with the highly charged groups in the amino acids and proteins.

The values found for the density of unfractionated casein in the dry state and in solution (Table V) are considerably lower than the corresponding values reported by Chick and Martin.<sup>8</sup> It appears unlikely that the presence of air in the casein could account for the present finding of a lower density in the solid state, since air was removed from the casein before placing the sample in the gradient tube. The use of centrifugation in determining the density also reduces the probability of error due to adhering air. The values obtained for the densities of the components of casein in the solid state and in solution are consistent with their amino acid compositions as well as the density of unfractionated casein.<sup>24</sup>

The contraction in volume for the proteins shown in Table V is remarkably constant, with an average value of 0.046 ml. per gram protein, or about 6%. The change in volume due to electrostriction by the charged groups has been calculated by assuming

(27) E. J. Cohn, *Ann. Rev. Biochem.*, **4**, 93 (1935).

that each pair of charged groups causes a contraction of 20 cc.<sup>28</sup> This effect accounts for about one-half the total electrostriction, varying somewhat with the protein. It is apparent, however, that the volume contraction of the caseins on dissolving is not proportional to the number of pairs of charged groups. Linderstrøm-Lang<sup>29</sup> has shown that the contraction in volume due to the splitting of peptide bonds amounts to as much as 50 ml. per mole of peptide group during the initial hydrolysis of proteins by enzymes, as compared with the normal value of 20 cc. for most peptide groups. This may be taken as an indication of the possibility that some charged groups have a greater effect of electrostriction than the usual value of 20 cc./mole for each pair. The source of the effect of this contraction has not been determined, but it may be due to a collapse of the molecular structure of the protein rather than an abnormal contraction due to the charged groups.

PHILADELPHIA 18, PA.

(28) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 378.

(29) K. Linderstrøm-Lang, *Cold Spring Harbor Symposia on Quant. Biol.*, **14**, 117 (1950).