Proteose-Peptone Fraction of Bovine Milk: Lacteal Serum Components 5 and 8—Casein-Associated Glycoproteins

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Abstract

Lacteal serum Components 5 and 8 were isolated from both heated and unheated skim milk. At pH 8.6 Component 5 migrated as a double zone in starch-urea-gels (discontinuous buffer) and as a single zone in polyacrylamide gels (continuous buffer). Component 8 migrated in the ion front on starch gels but resolved into two principal zones in the polyacrylamide gel system, designated as 8-fast and 8-slow in ascending order of relative mobility. Components 8-fast and 8-slow were fractionated by gel-permeation chromatography on Bio-Gel P-10 polyacrylamide beads.

Component 5 is characterized by its high proline (~10.5%) and relatively low carbohydrate contents (~1.5%). Components 8-fast and 8-slow contain relatively high concentrations of total phosphorus (~7.4 and 5.5% as \( \text{H}_2\text{PO}_4 \)) and intermediate amounts of carbohydrate (~2.07 and 9.0%). All three components are void of cysteine and cystine and contain only low concentrations of methionine.

In veronal buffer at pH 8.6, \( \gamma /2 = 0.1 \), a sedimentation coefficient \( (S^\circ_{20,w}) \) of 1.22 and a molecular weight \( (M_w) \) of ~14,300 were observed for Component 5. Similar parameters for Components 8-fast and 8-slow were \( S^\circ_{20,w} = 4,100 \) and 9,900. An isoelectric pH of 3.3 is reported for Component 8-fast and partial specific volumes \( (V) \) of 0.736, 0.700, and 0.719 (cc/g) for Component 5, 8-slow, and 8-fast were calculated from composition data.

The "proteose-peptone" fraction of skim milk has been characterized as a mixture of heat-stable, acid-soluble glycoproteins (5, 6, 10, 11, 13, 18, 19, 22, 24, 27). Classically, these proteins have been detected in and isolated from the filtrate of heated skim milk (95 C for 30 min) following adjustment to pH 4.6 to 4.8. However, in recent times, this fraction and components thereof have been identified in and isolated from unheated skim milk (2, 6, 14, 18, 22). Thus, a sometimes stated hypothesis that the proteose-peptone fraction consisted of "secondary" or derived proteins is refuted. Apparently the stability of these proteins to heat and acid led investigators to conclude that they were confronting much smaller molecules.

Larson and Rolleri (19) observed three discernible boundaries in Tiselius patterns of the proteose-peptone fraction of heated skim milk designated as milk serum Components 3, 5, and 8 in ascending order of electrophoretic mobilities. Jenness (13, 14) isolated a relatively pure preparation of Component 5 from unheated skim milk by its coprecipitation with casein when NaCl was added to saturation. Interestingly, he noted that unheated preparations exhibited a loaf-depressant property when incorporated into bread doughs and that heating the preparation negated this property. Further, he suggested that Component 5 was the principal component of the proteose-peptone fraction and that it was unlike other milk proteins. Ganguli et al. (11) separated a "proteose" from the proteose-peptone fraction by salting-out with \( (\text{NH}_4)_2\text{SO}_4 \) when added to one-half saturation. Because of similarities in the fractionation procedures, the proteose thus obtained should be comparable to the sigma-proteose fraction reported earlier by Aschaffenburg (1). Extensive composition data were reported for the proteose-peptone fractions; their glycoproteinaceous nature was confirmed.

Aschaffenburg and Drewry (2) detected 6 zones on paper electropherograms of proteose-peptone fractions prepared from both heated and unheated skim milk. They concluded that the principal and fastest migrating zone corresponded to Component 5 and that the 5 slower zones were constituents of Component 3. No indication was given relative to zones corresponding to Component 8. Previous reports from this laboratory described the preparation of proteose-peptones from both heated and unheated skim milk as well as the distribution of these proteins within the milk protein systems.

Received for publication March 2, 1970.

1 Published as Journal article no. 5011 from the Michigan Agricultural Experiment Station.
2 Present address: Ralston-Purina Corp., St. Louis, Missouri.
PROTEOSE-PEPTONE PREPARATIONS

[Obtained from heated or unheated skim milk (22)]

Make up to 1 to 2% in deionized water
Adjust to pH 7.0
Add (NH₄)₂SO₄ to 25% saturation
Adjust to pH 4.9
Centrifuge at 8,000 × g for 30 min

SUPERNATANT S₁

Add (NH₄)₂SO₄ to 40% saturation
at pH 4.9
Centrifuge at 8,000 × g
for 30 min

RESIDUE R₁
(Enriched in Component 5)

Disperse in water at pH 7.0
Centrifuge at 1,000 × g for 30 min,
discard pellet
Repeat salting-out step 4 ×
at pH 4.6
Disperse in water and desalt on
Bio-Gel P-2
Lyophilize and dry over P₂O₅

COMPONENT 5

SUPERNATANT S₂

Adjust to pH 7.0
Add (NH₄)₂SO₄ to 50% saturation
at pH 7.0
Centrifuge at 8,000 × g
for 30 min

RESIDUE R₂
(Components 3 and 5)

COMPONENT 3

SUPERNATANT S₃

Add (NH₄)₂SO₄ to 65% saturation
at pH 7.0
Centrifuge at 8,000 × g
for 30 min

RESIDUE R₃
(Enriched in Component 3)

COMPONENT 3

SUPERNATANT S₄

Add (NH₄)₂SO₄ to 80% saturation
at pH 7.0
Centrifuge at 8,000 × g
for 30 min

RESIDUE R₄
(Components 3 and 8)

COMPONENT 8-FAST

SUPERNATANT S₅

Concentrate and fractionate
on Bio-Gel P-10
Desalt fractions on Bio-Gel P-2
Lyophilize and dry over P₂O₅

RESIDUE R₅
(Discard)

COMPONENT S-SLOW

Fig. 1. Schematic representation of the procedures employed to fractionate proteose-peptone preparations into Components 3, 5, 8-fast, and 8-slow.

JOURNAL OF DAIRY SCIENCE VOL. 53, NO. 8
Components 5 and 8 were isolated from both the whey and casein protein fractions, whereas Component 3 was found only in the whey fraction and was characterized by a carbohydrate content of about 17%.

It seems appropriate to note the excellent studies of Bezkorovainy (3, 4), who isolated several phosphoglycoprotein species from bovine serum and the acid wheys of both cow's milk and colostrum by a combination of DEAE and CMC-cellulose chromatography. The analytical data reported for these glycoproteins were qualitatively similar to those of the proteose-peptone fractions (6, 11, 27).

The purpose of our study was to provide basic chemical and physical characteristics of the proteose-peptone components usually identified as milk serum Components 5 and 8. A preliminary report of the principal results was presented previously (17).

**Materials and Methods**

Fresh, uncooled milk from the University dairy herd (Holstein cattle) was centrifugally separated to obtain the skimmilk portion from which all protein preparations were derived. The Component 5 preparation characterized in this study was isolated from heated (95°C for 30 min) skimmilk, whereas preparations of Component 8 were obtained from both heated (95°C for 30 min) and unheated skimmilk.

**Preparation of Components 5 and 8**

The basic procedure employed to fractionate proteose-peptone into its individual components is outlined in Figure 1. As indicated, the isolation process begins with a proteose-peptone preparation from heated skimmilk or a proteose-peptone-enriched preparation from unheated skimmilk. Procedures for obtaining these preparations were described in a report from this laboratory (22). Fractionation of the proteose-peptones was by salting-out at selected concentrations of (NH$_4$)$_2$SO$_4$ at controlled levels of pH. Residue-R$_1$, a Component 5-rich precipitate, was further purified by repeated solution, centrifugation, and salting-out at 25% (w/v) saturation with (NH$_4$)$_2$SO$_4$. Residues R$_2$, R$_3$, and R$_4$ represent proteose-peptone fractions salted out with (NH$_4$)$_2$SO$_4$ at concentrations of 40, 50, and 65%, respectively, and contain varying proportions of Components 3, 5, and 8. Residue-R$_3$ consists essentially of Component 3, a whey glycoprotein (22).

Supernatant-S$_5$, containing essentially the proteins attributed to Component 8, was sub-

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**Fig. 2.** Starch urea gel electropherograms of proteose-peptone preparations from unheated and heated skimmilk. The preparation from unheated skimmilk shows the presence of contaminating whey proteins.
fractionated into two principal fractions—i.e., 8-fast and 8-slow—by gel permeation chromatography on polyacrylamide beads. Glass columns (2.8 cm id and 40 cm long) were coated with dimethyldichlorosilane and filled with water-equilibrated, Bio-Gel P-10 polyacrylamide beads. A void volume of approximately 55 ml and a flow rate of about 150 ml/hour characterized the column operations. Five milliliters of Component 8-containing solution, concentrated to about 2% (w/v) protein, were added to the top of the column and eluted with deionized water. The eluate was monitored at 254 and 280 nm. Eluate fractions were collected at 25, 30, 40, and 50 ml, measured from the point of emergence of the initial peak. Each fraction was assayed by polyacrylamide gel electrophoresis to identify the fractions containing unresolved protein species. These fractions were combined and rechromatographed to achieve further separation of the principal components.

Salt-free, lyophilized preparations of Components 5 and 8, suitable for subsequent chemical and physical analyses, were obtained by passing 2% (w/v) solutions of the protein preparations over Bio-Gel P-2 columns, followed by lyophilization and exposure to P2O5 in a partial vacuum for 48 hours. The common technique of exhaustive dialysis to eliminate salt ions from protein systems was untenable for these proteins because of their molecular size. Up to one-half of the proteinaceous material passed through a Visking membrane in 24 hours.

**Characterization of Components 5 and 8**

*Amino acids.* Amino acid compositions of the protein preparations were determined on 20- and 70-hour acid hydrolysates (0.6 mg/ml of 6 N HCl at 110 C). Analyses were performed with a Beckman-Spinco Model 120-C Automatic Amino Acid Analyzer. Chromatograms were quantitated by comparison with a standard amino acid mixture. Nor-leucine was employed as an internal standard. All residue concentrations were corrected to reflect their maximum values (12). Tryptophan and cysteine contents were evaluated by the methods of Spies (25) and Ellman (8).

*Carbohydrates.* Components of the carbohydrate moiety were identified, as described (22), by paper chromatography following their re-
lease from the protein by mild hydrolysis in 2 N HCl. Authentic glucosamine, galactosamine, galactose, glucose, mannose, and fucose were used as reference materials for tentative identifications. Chromatograms were migrated with a butanol-pyridine-water (6:4:3, v/v) mixture by descending chromatography. A solution of triphenyltetrazolium with NaOH was used for visualization of carbohydrate-containing zones. Qualitative identification of glucosamine and galactosamine was augmented by their identification in chromatograms from the amino acid analysis.

Quantitative estimates of the carbohydrate classes were achieved by spectrophotometric techniques. Hexose was determined by the phenol–sulfuric acid method of Dubois et al. (7). Hexosamine was determined by the method of Johansen et al. (15) in which the chromogen resulting from addition of acetylacetone was steam-distilled into Ehrlich's p-dimethylaminobenzaldehyde receiver. Sialic acid was determined by Warren's (30) thiobarbituric acid method as modified by Marier et al. (20).

Elemental analyses. Protein nitrogen was determined by a micro-Kjeldahl procedure. Phosphorus was determined by the method of Sumner (26). Electrophoretic methods. Electrophoretic mobilities of the protein specimens were evaluated from Tiselius patterns with a Perkin-Elmer Model 38-A Electrophoresis Apparatus, employing a 2-ml cell. The protein specimens were carried in veronal buffer of pH 8.6 and 0.1 ionic strength. One specimen of Component 8 (i.e., 8-fast) was observed from pH 2.0 to 8.6. Mobilities were plotted versus pH to estimate its isoelectric pH. The concentration of protein was 15 mg/ml for all electrophoretic runs.

A starch urea gel (SUG) zonal electrophoretic technique was adapted from the procedure described by Wake and Baldwin (29). Electrophoresis was performed in a water-cooled gel bed at 15°C. The gel was connected to one-liter buffer tanks with paper-filter wicks (E and D 652). The usual discontinuous buffer system, consisting of Tris-citrate-urea (pH 8.6) in the gel and boric acid-NaOH (pH 8.6) in the buffer tanks, was employed.

Polyacrylamide gel (PAG) electrophoresis was performed in both horizontal (laboratory-constructed) and vertical (E-C Apparatus Co.) water-cooled gel beds. A continuous buffer system (pH 8.6), consisting of boric acid (13.0 g/liter) and NaOH (2.1 g/liter), was employed in these applications. Cyanogen 41, at an 8% concentration in the borate buffer and catalyzed with 0.03 ml/liter of N, N, N1, N1-tetramethyl-ethylenediamine and 0.3 g/liter of ammonium persulfate, served as the gel medium. Urea was added to 4.5 M when its incorporation into the acrylamide gel was desired. Electrophoresis in the horizontal gels was performed at a constant current of approximately 15 mamp/cm² of cross-sectional area for about 15 hours. In the vertical E-C cell, electrophoresis was performed at a constant cell current of about 100 mamp for 6 hours.

 Routinely, the resolved protein-containing zones were visualized by the Amido Black staining procedure. In one set of experiments, involving the identification of glycoproteins in PAG patterns of proteose-peptone and related fractions, a periodic acid–Schiff reagent was used (16).

Ultracentrifugal techniques. Sedimentation-velocity and sedimentation-equilibrium studies were performed in a Beckman-Spinco, Model E Analytical Ultracentrifuge. A capillary-type, synthetic-boundary cell operated at 59,780 rpm.
and 20 C was employed for the sedimentation-velocity studies. Protein concentrations ranging from 5 to 10 mg/ml in veronal buffer (pH 8.6, \( \Gamma/2 = 0.1 \)) were employed. Apparent-sedimentation coefficients were corrected for solvent effects and protein concentrations and expressed as \( S_{20, w}^c \) at infinite dilution of the protein.

A short-column, sedimentation-equilibrium technique by Van Holde and Baldwin (28) was employed for determination of molecular weights. Analyses were performed at 20 C and at speeds of about 25,000 rpm in a double-sector, synthetic-boundary cell fitted with a filled-Epon centerpiece. Apparent weight-average molecular weights of protein of from 5 to 10 mg/ml in veronal buffer were plotted versus protein concentration and extrapolated to a standard value at infinite dilution of the protein (i.e., \( M^w_\infty \)).

Solvent densities were determined with 10-ml pycnometers equilibrated to 20 ± .01 C. Solution densities were calculated according to Fujita (9) from the expression \( \rho_{\text{soln}} = \rho_{\text{solv}} + (1 - \frac{\rho_{\text{solv}}}{\rho_{\text{soln}}}) \rho_{\text{solv}} \), where \( \rho \) is the concentration of the protein in g/ml. Relative viscosities were determined in a Cannon-Ubbelohde semimicroviscometer at 20 ± 0.01 C and partial specific volumes (\( \bar{V} \)) were calculated from the compositional data (Table 1).

**Results and Discussion**

**Preparation of Components 5 and 8 from Heated and Unheated Skimmilk**

Starch urea gel electropherograms of proteose-peptone-rich preparations obtained from heated and unheated skimmilk are shown in Figure 2. These patterns are essentially similar to data reported by Kolar and Brunner (18). Component 8 migrated as a single zone in the ion front, whereas Component 5 appeared as a double zone in the middle of the pattern and Component 3 was a single dominant zone of slower mobility. The preparation from unheated skimmilk contained other whey protein contaminants.

The fractionation of these preparations into Components 3, 5, and 8 by the scheme outlined in Figure 1 was monitored by both horizontal
Table 1. Composition of Component 5, 8-fast, and 8-slow specimens isolated from heated and unheated skimmilk.

<table>
<thead>
<tr>
<th>Component</th>
<th>Component 5</th>
<th>Component 8-fast</th>
<th>Component 8-slow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heated</td>
<td>Heated</td>
<td>Unheated</td>
</tr>
<tr>
<td>Lysine</td>
<td>7.55</td>
<td>5.52</td>
<td>7.92</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.92</td>
<td>0.82</td>
<td>1.44</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.60</td>
<td>5.42</td>
<td>3.63</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>5.81</td>
<td>8.11</td>
<td>8.20</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.72</td>
<td>5.04</td>
<td>4.48</td>
</tr>
<tr>
<td>Serine</td>
<td>5.77</td>
<td>10.00</td>
<td>12.17</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>24.39</td>
<td>25.30</td>
<td>23.96</td>
</tr>
<tr>
<td>Proline</td>
<td>10.55</td>
<td>3.67</td>
<td>2.51</td>
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<td>Glycine</td>
<td>1.23</td>
<td>1.19</td>
<td>1.21</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.92</td>
<td>0.93</td>
<td>0.80</td>
</tr>
<tr>
<td>1/2 Cystine</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Valine</td>
<td>6.60</td>
<td>5.18</td>
<td>5.57</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.68</td>
<td>1.10</td>
<td>1.07</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>6.14</td>
<td>7.36</td>
<td>8.41</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.33</td>
<td>7.16</td>
<td>4.27</td>
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<tr>
<td>Tyrosine</td>
<td>1.94</td>
<td>0.70</td>
<td>1.45</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.86</td>
<td>1.04</td>
<td>0.73</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.20</td>
<td>0.23</td>
<td>0.06</td>
</tr>
<tr>
<td>Cysteine c</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Unidentified</td>
<td></td>
<td>0.47</td>
<td>0.18</td>
</tr>
</tbody>
</table>

| Waa a     | 95.71       | 88.72            | 88.06            | 77.19            | 82.17            |
| Hexose    | 0.93        | 1.37             | 0.99             | 4.53             | 2.98             |
| Hexosamine| 0.23        | 0.26             | 0.35             | 2.45             | 1.79             |
| Sialic Acid | 0.29        | 0.40             | 0.47             | 3.29             | 2.88             |

| W CHO      | 1.45        | 2.03             | 1.81             | 10.27            | 7.63             |
| Phosphorus (H₂PO₃) | 2.53 | 6.26             | 8.59             | 5.17             | 5.79             |
| W total    | 99.69       | 97.01            | 98.46            | 92.63            | 95.61            |
| Nitrogen   | 13.83       | 13.33            | 13.16            | 12.31            | 13.03            |

a Amino acid residues were determined from 20- and 70-hour hydrolysates (6 N HCl) and corrected for time of hydrolysis according to Hirs et al. (12).

b Method of Spies (25).

c Method of Ellman (8).

SUG and PAG electrophoresis (Fig. 3). The final supernatant-S₅ contained materials which migrated in a sharp zone with the ion front in starch gels run with the discontinuous buffer system, but showed two principal and several minor zones in polyacrylamide gels run with the continuous borate buffer system. When the Component 8-rich fraction was subjected to electrophoresis in PAG, a second dimension—i.e., normal to the original direction—with the discontinuous buffer system, both of the principal zones moved with the ion front as a single zone. Thus, it was concluded that Component 8 was a mixture of at least two principal proteins, designated here as 8-fast and 8-slow in descending order of their rates of electrophoretic migration. The purified preparation of Component 5 (Residue R₅ in Fig. 1) exhibited two closely migrating zones by SUG electrophoresis and but one principal zone by PAG electrophoresis. However, because of the relatively high concentration (4% soln) of sample applied to the PAG, resolution of these proteins may have been obscured. And, as reported previously, preparations of Component 3 (Residue R₃ in Fig. 1) showed a single zone in both SUG and PAG, employing both continuous and discontinuous buffer systems, electrophoresis when stained with Amido Black.

Polyacrylamide gel (continuous) electrophoretic patterns of the 5 eluate fractions collected during the gel filtration (Bio-Gel P-10) of the
Component 8-containing Supernatant-S₁₅ are shown in Figure 4. The first two fractions were predominantly Component 8-slow, whereas the last fraction contained Component 8-fast. The third and fourth fractions were composed of a mixture of 8-fast and 8-slow and were rechromatographed to yield the individual components.

A proteose-peptone preparation and selected fractions obtained therefrom by the scheme outlined in Figure 1 were subjected to vertical (E-C cell) PAG electrophoresis in a continuous buffer system. Figure 5 shows two sets of electropherograms: one was stained with Amido Black to show the zones of migrating proteins; whereas, the second was treated with periodic Schiff reagent to show zones of glycoproteins. In these patterns, Component 5 stained heavily with Amido Black but was barely visible when treated for the detection of glycoproteins. Similarly, Components 8-fast and 8-slow were more visible in the Amido Black-stained gel but showed additional zones in the carbohydrate-developed gel. Component 3, although clearly visible in both gels, constituted the predominant glycoprotein zone.

COMPOSITIONAL CHARACTERISTICS OF COMPONENTS 5 AND 8

The amino acid, carbohydrate, phosphorus, and nitrogen compositions of proteose-peptone Components 5, 8-fast, and 8-slow are reported in Table 1. These proteins have common characteristics of low methionine content and the absence of cysteine and cystine. Component 5 contains significantly more proline (~10.5%) than either of the Component 8 fractions. Indeed, the proline and glutamic acid contents of Component 5 are quite similar to those reported for casein. The three proteose-peptone proteins contain about equal concentrations of aspartic acid plus glutamic acid (~30%). However, the distribution of these residues varies between
the three species. The amino acid compositions reported are in general agreement with compositions reported by Bezkorovainy (4) for several lacteal phosphoglycoproteins, but they are quite dissimilar to those reported by Ganguli et al. (10) for a proteose-peptone preparation. They (10) reported high values for cysteine (50%), methionine (5.0%), and tryptophan (4.2%), suggesting the possibility that the principal whey proteins were contaminants in their preparation.

The total carbohydrate contents of Components 5, 8-fast, and 8-slow were approximately 1.5, 2.0, and 9.0%. The carbohydrate content of Component 3 was reported to be about 17% (22). This compositional feature reflects the results observed in the polyacrylamide gel developed for glycoproteins in which Component 3 was the dominant zone. The chromatographic studies demonstrated that sialic acid, galactose, mannose, galactosamine, and glucosamine were constituents of Components 5, 8-fast, and 8-slow but their relative compositions varied between proteins.

The low nitrogen contents of these protein fractions (~ 12 to 14%) reflect the presence of carbohydrate moieties and the relatively high concentrations of phosphorus (0.97% for Component 5, 1.98 to 2.22% for Component 8-slow, and 2.40 to 3.29% for Component 8-fast). The analyses as presented in Table 1 account for approximately 99.7% of Component 5, 92.6 to 95.6% of Component 8-slow, and 97.0 to 98.5% of Component 8-fast. Based on the compositional data reported, partial specific volumes (\(\bar{V}\)) of 0.736 cc/g for Component 5, 0.700 cc/g for Component 8-slow, and 0.719 cc/g for Component 8-fast were calculated.

### Physical Properties

Tiselius electrophoretic patterns of Components 5, 8-slow, and 8-fast in veronal buffer at pH 8.6, \(\Gamma/2 = 0.1\), showed single, rather diffuse boundaries with average mobilities of \(-4.9\), \(-9.2\), and \(-9.2\) Tiselius units (Fig. 6). These mobilities are slightly higher than those reported previously for the proteose-peptone components in a mixed system, i.e., proteose-peptone or fractions therefrom (15, 19). This observation is rationalized by assuming some degree of molecular interaction between the individual components comprising the proteose-peptone system. Supporting this conjecture was the observation that purified specimens of Component 5 precipitated from a saline solution at pH 4.8, whereas a solution of proteose-peptone at the same conditions remained stable. Obviously, Components 8-fast and 8-slow cannot be distinguished by moving-boundary electrophoresis. An isoelectric pH of 3.3 (\(\mu = 0\)) at an ionic strength of 0.1 was determined for Component 8-fast from a plot of electrophoretic

<table>
<thead>
<tr>
<th>Component</th>
<th>Protein (\alpha)</th>
<th>(S_{20,w}^{app})</th>
<th>(M_{w,app})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component 5 (b)</td>
<td>4.1</td>
<td>1.21</td>
<td>13,700</td>
</tr>
<tr>
<td></td>
<td>5.1</td>
<td>1.19</td>
<td>13,400</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>1.21</td>
<td>(\ldots)</td>
</tr>
<tr>
<td></td>
<td>6.1</td>
<td>1.19</td>
<td>13,400</td>
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<td></td>
<td>(S_{20,w}^{app} = 1.22)</td>
<td>(M_{w,app} = 14,300)</td>
<td></td>
</tr>
<tr>
<td>Component 8-fast (c)</td>
<td>5.2</td>
<td>0.74</td>
<td>4,000</td>
</tr>
<tr>
<td></td>
<td>6.1</td>
<td>(\ldots)</td>
<td>4,100</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>0.73</td>
<td>3,500</td>
</tr>
<tr>
<td></td>
<td>10.1</td>
<td>0.70</td>
<td>3,500</td>
</tr>
<tr>
<td></td>
<td>(S_{20,w}^{app} = 0.78)</td>
<td>(M_{w,app} = 4,100)</td>
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<tr>
<td>Component 8-slow (c)</td>
<td>5.0</td>
<td>1.27</td>
<td>8,700</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>1.16</td>
<td>7,100</td>
</tr>
<tr>
<td></td>
<td>10.1</td>
<td>1.16</td>
<td>7,000</td>
</tr>
<tr>
<td></td>
<td>(S_{20,w}^{app} = 1.35)</td>
<td>(M_{w,app} = 9,900)</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) In veronal buffer, pH 8.6, \(\Gamma/2 = 0.1\).
\(b\) Obtained from heated skimmilk.
\(c\) Obtained from unheated skimmilk.
mobilities at various values of pH (pH 2.0 to 8.6).

An interesting phenomenon was encountered when Component 8-fast was dissolved (15 mg/ml) in veronal buffer in preparing the free-boundary experiments. After approximately 2 hours of dialysis against the buffer, a dense precipitate was formed which appeared as irregular rhombic crystals under a light microscope at 26 magnification (Fig. 7). Crystals of sodium veronal formed by evaporating a portion of the buffer were needle-like, with no resemblance to the crystals harvested from the protein-veronal solution. When observed in the presence of a small amount of crystal violet solution, the crystals appeared to take up the dye. Thus, it is suggested that their crystals consisted entirely of protein or at least a protein adjunct of sodium veronal. This phenomenon was not observed with either Components 5 or 8-slow.

Sedimentation coefficients and weight-average molecular weights for Components 5, 8-slow, and 8-fast are presented in Table 2. Photographic reproductions of typical ultracentrifugation data from which these values were calculated are shown in Figure 8. These data indicate that Components 5, 8-fast, and 8-slow are relatively small proteins possessing molecular weights of approximately 14,300, 4,100, and 9,900 Daltons. Corresponding $S_{20,w}$ values of 1.22, 0.78, and 1.23 are reported. Ogston (23) reported sedimentation coefficients of 0.96 and 2.75 for two components observed in Aschaffenburg's sigma-protease. Molecular weights of 4,900 and 23,900, respectively, were reported. Similar sedimentation coefficients ($S_{20,w}$) were observed by Brunner and Thompson (6) for the two principal sedimentating species in proteose-peptone and its partial fractions.

Because of the observation that Component 5 showed two zones in starch-urea, it is suggested

![Fig. 8. Representative ultracentrifugal data characterizing the proteose-peptone fractions: A, Component 8-fast from unheated skim milk; B, Component 8-slow from unheated skim milk; and C, Component 5 from heated skim milk. All determinations were made in veronal buffer at pH 8.6, F/2 = 0.1 and at 50 C. Sedimentation-velocity patterns represent trials at 59,780 rpm with protein concentrations of 7.0, A; 7.1, B; and 6.1 C mg/ml. Sedimentation-equilibrium patterns represent trials at about 23,000 rpm for the protein concentrations indicated.](image-url)
that this component consists of two protein species, differing merely in simple amino acid substitutions (polymorphs) which sediment similarly, or that they represent two smaller proteins which sediment as a dimer. Obviously, more detailed studies are indicated before the complex nature of the proteose-peptone system can be described.

**DISTRIBUTION OF COMPONENTS 5 AND 8 IN THE MILK PROTEIN SYSTEM**

Serum Components 5 and 8, together with Component 3, are unique members of the milk protein system. They constitute the principal constituents of the proteose-peptone fraction isolated from whey and, to a lesser degree, the casein complex. The distribution of Components 5 and 8 differs from that of Component 3, which does not constitute a part of the casein system (18, 22). Therefore, should Components 5 and 8 be classified as caseins since they are recovered, in part, from skimmilk under the same conditions casein is obtained? Their relatively high concentration of phosphorus and, in Component 5 an equivalent concentration of proline suggest similarities with casein. And, like θ-casein, they contain carbohydrate moieties. Furthermore, when skimmilk is saturated with NaCl, Component 5 is recovered as the principal proteose-peptone in the casein complex. Also, it has been noted that Components 5 and 8 are gradually released from the casein complex by repeated isoelectric precipitations of the casein or by a relatively mild heat treatment (95°C for 30 min). Moreover, it is of interest that the yields of Components 5 and 8 are greater, as components of the proteose-peptone fraction, when isolated from heated than from unheated skimmilk.

The functions, if any, of Components 5 and 8 as constituents of the casein complex are unknown. The observations that they occur in both the casein and whey fraction suggests they exist in a state of dynamic equilibrium between association with casein and in solution. Based upon the observations reported in this study and other general properties already mentioned, we tentatively designate milk serum Components 5 and 8 constituents of the classical proteose-peptone system as casein-associated glycoproteins.

**References**


