THE PROTEINS OF BOVINE SEMINAL PLASMA

1. PRELIMINARY AND ELECTROPHORETIC STUDIES

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(Received for publication, August 17, 1953)

The protein constituents of seminal plasma long have been considered of importance in the maintenance of proper conditions for spermatozoa. In fact, in the practice of artificial insemination, various protein-containing systems such as milk, gelatin solution, and egg yolk, in addition to various chemical buffer systems, have been used as semen extenders (1-5).

Since ejaculated seminal plasma is a composite of the products of several of the accessory organs and glands of the male reproductive system, it might be expected that the proteins present would be of a heterogeneous mixture. Apparently little work has been done on the characterization of the seminal plasma proteins of any species other than the human.

Several investigators have analyzed the proteins of human seminal plasma. Huggins et al. (6, 7) found that only traces to 18 per cent of the human seminal plasma proteins were heat-coagulable both at neutrality and at pH 5.5; approximately 60 per cent of the proteins were of a proteose nature and dialyzable. The undialyzable proteins corresponded electrophoretically to the α- (21.3 per cent), β- (39.6 per cent), and γ- (19.1 per cent) globulins and the serum albumin (19.9 per cent) of blood. Ross et al. (8-10) also examined the human seminal plasma proteins by similar means and concluded that, although proteoses, blood globulins, and glycoproteins were present in quantity, there was little serum albumin.

This study was undertaken to characterize the proteins of bovine seminal plasma.

Methods

Semen was collected from healthy bulls of the University herd, carefully cooled, and transported immediately to the laboratory for analysis. The seminal plasma was obtained by centrifuging the whole semen at 2000 \( \times g \) for 10 minutes and decanting the clear supernatant plasma from the packed sperm cells. Nitrogen analyses by a semimicro-Kjeldahl procedure (11) showed that the amount of protein in seminal plasma varied between 3 and 8 per cent; there was a marked tendency for the plasma from the older bulls to have the higher values. For electrophoretic analysis, concentrated buffer was added to the seminal plasma to bring both to ap-
proximately the proper concentration. This solution was equilibrated in Visking cellulose casing (with a few drops of toluene) for 12 hours at 4° with frequent agitation against buffer 30 to 50 times the volume of the solution and then for 12 to 24 additional hours against a fresh change of the same amount of buffer. Throughout this investigation, a sample of seminal plasma pooled from several bulls of various ages and stored at −18° was used for the chemical analyses.

Solutions of the bovine blood serum proteins were prepared by treating the clear serum removed from clotted blood in the same manner as for the seminal plasma proteins.

Buffers of known pH and ionic strength were prepared according to the specifications of Miller and Golder (12) for the glycine-NaCl-HCl buffer, of Green (13) for the phosphate buffers, and of Longsworth (14) for the Veronal buffer.

Electrophoretic analyses were conducted at 1.2° in an American Instrument Company portable electrophoresis apparatus equipped with a cylindrical lens and rotating slit. Since the major electrophoretic components of the bovine seminal plasma possess mobilities close to each other, mobilities were calculated from the more distinct patterns of the ascending limb by correlating them as closely as possible with those of the descending limb. The areas under the curves were projected and evaluated with a planimeter according to the procedure of Tiselius and Kabat (15) to approximate the relative concentration of the components.

Results

Chemical Studies—To determine the amount of dialyzable nitrogen present in the bovine seminal plasma, known amounts of the pooled sample were exhaustively dialyzed in Visking cellulose casing against frequent changes of cold phosphate buffer (pH 6.9, ionic strength of 0.1). Analysis of this seminal plasma sample indicated that originally there were 9.41 mg. per ml. of nitrogen present of which 8.38 mg. per ml. were precipitable by 12 per cent trichloroacetic acid. After 7 days of dialysis, 8.25 mg. per ml. of nitrogen (corrected basis) were still recoverable. Previous work (16) has shown that about 20 per cent of the dialyzable or non-trichloroacetic acid-precipitable nitrogen present is accounted for by free amino acids, which are responsible for all of the major ninhydrin color-producing substances.

Preliminary results in this investigation indicated that heating the bovine seminal fluid at neutrality produced considerable turbidity, and, when diluted with an equal volume of 1.3 N acetate buffer at pH 4.6, the plasma formed a thick gel at 98°. Thus, the pooled sample of seminal plasma was diluted with 2 volumes of phosphate buffer (pH 6.9, ionic strength of 0.1) to a total concentration of about 3.8 mg. of nitrogen per ml. When
heated for 1 hour at 98° with an equal volume of 0.15 N acetate buffer at pH 4.6, 76 per cent of the proteins present were coagulated, 13 per cent remained in solution, and 13 per cent appeared in the fraction non-precipitable by 12 per cent trichloroacetic acid. About half of the coagulated proteins were precipitated upon the addition of the acetate buffer and the rest during the heat treatment. When a 0.75 N acetate buffer at pH 4.6 was used under similar conditions, no precipitate formed upon its addition; 46 per cent of the proteins were coagulated on holding at 98° for 1 hour and 12 per cent of the original proteins appeared in the fraction non-precipitable by 12 per cent trichloroacetic acid.

Thus, when compared with the results of Huggins et al. (6, 7) and Ross et al. (8–10), the seminal plasma proteins of the bovine differ from those of the human in being less dialyzable and more heat-coagulable. The observations reported in the present investigation apply to that approximate 90 per cent of the nitrogen present in the seminal plasma which is non-dialyzable and considered to be a part of what is called the bovine seminal plasma proteins.

For determining the nitrogen content of the seminal plasma proteins, pooled seminal plasma was exhaustively dialyzed for 4 days against cold distilled water, freeze-dried under a vacuum, placed over P₂O₅ for several days, extracted with dry ether for 24 hours, and dried at 100° for 2 hours. Analysis of the material, which was readily soluble in neutral buffer, indicated a nitrogen content of 14.38 per cent as determined by the Kjeldahl procedure. A second sample was exhaustively dialyzed, and moisture and Kjeldahl determinations were made on the suspension, indicating a nitrogen content of 14.32 per cent.

These figures on the nitrogen content of the bovine seminal plasma proteins seem to be low, since most proteins contain around 16 per cent nitrogen, and numerous studies have shown that semen contains a high content of the basic amino acids. Sarkar et al. (17) have found, however, that, while dried bovine sperm cells contained 17.6 per cent nitrogen, the dried seminal plasma contained only 12.1 per cent nitrogen, both corrected for fat and ash content but not for the dialyzable organic constituents. Inasmuch as a Molisch test of exhaustively dialyzed seminal plasma is quite weak, it would appear that non-dialyzable carbohydrate constituents are present in low concentration. Even if the dialyzable organic constituents are taken into consideration (chiefly fructose (1–3)), it is apparent that the value obtained by Sarkar et al. (17) also indicates a low nitrogen content for the seminal plasma proteins. In addition, these investigators determined the concentration of eleven of the amino acids in dried bovine seminal plasma and sperm cells and found that the basic amino acids were not relatively prevalent in the former but were quite high in the latter.

Electrophoretic Behavior in Various Buffers—In Fig. 1 are presented elec-
trophoretic patterns of the pooled sample of the bovine seminal plasma proteins in several buffers of varied pH and ionic strength. Since solutions of the seminal plasma proteins became turbid at approximately pH 3.0 to

![Electrophoretic Analysis of Bovine Seminal Plasma Proteins in Several Buffers](image)

**Fig. 1.** Electrophoretic analysis of a pooled sample of the bovine seminal plasma proteins in several buffers. A, glycine-HCl-NaCl, ionic strength 0.1; B, K phosphate, ionic strength 0.2; C, K-Na phosphate, ionic strength 0.1; and D, Na Veronal, ionic strength 0.1. Analysis times were 9000, 13,800, 5400, and 5400 seconds, field strengths 2.6, 3.3, 6.7, and 7.8 volts per sq. cm., and protein concentrations 2.2, 2.2, 2.0, and 2.0 per cent respectively. See Table I.

### Table I
**Electrophoretic Analysis of Bovine Seminal Plasma Proteins in Several Buffers**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Component No.</th>
<th>Mobility† and per cent of component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1  2  3  4  5  6  7  8  9  10 11</td>
</tr>
<tr>
<td>A Glycine, pH 2.02</td>
<td></td>
<td>+6.7 +9.7 +14.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16  76  8</td>
</tr>
<tr>
<td>B Phosphate, pH 5.89</td>
<td></td>
<td>+5.4 +1.2 +0.9 -1.0 -2.3 -3.7 -4.4 -4.6 -6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4  3  6  12  6  34  15  17  3</td>
</tr>
<tr>
<td>C Phosphate, pH 6.90</td>
<td></td>
<td>+4.1 -1.7 -3.1 -4.1 -5.2 -5.6 -6.3 -7.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4  8  13  21  32  17  5</td>
</tr>
<tr>
<td>D Veronal, pH 8.58</td>
<td></td>
<td>+3.1 -1.1 -2.5 -3.1 -4.4 -5.0 -5.3 -5.8 -6.4 -7.0 -7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4  2  11  3  20  27  29  2  2</td>
</tr>
</tbody>
</table>

*See Fig. 1 for identification of conditions and components.
†Mobilities in $1 \times 10^{-5}$ cm$^2$ volt$^{-1}$ sec.$^{-1}$.

5.5 unless the ionic strength was extremely high, electrophoretic patterns were not obtained for this range. The precipitability of the human seminal plasma proteins in this region also has been observed. Ross et al. (8) prepared some of the fractions in purified form by differential precipitation in this manner.
The electrophoretic patterns in Fig. 1 indicate the heterogeneous nature of the bovine seminal plasma proteins and the presence of three apparent major components and several minor ones. At least eleven components are discernible and probably several more are obscured by the more concentrated components. The mobilities of the various electrophoretic components and their relative concentration are presented in Table I. Since the identity of the individual components at the various pH values is not known, it should not be concluded that Component 3, for example, is the same material in all cases. It is noteworthy to observe that even at pH 8.6 a component was present which migrated toward the negative electrode (Component 1). The Veronal buffer of pH 8.6 with an ionic strength of 0.1 afforded the best electrophoretic separation of components with the least difficulties. Thus, it was used as the buffer medium in all further electrophoretic experiments.

Electrophoretic Behavior of Various Semen Samples—For this purpose
samples of semen from several bulls of various ages, breeds, and two successive ejaculations from the same bull were collected. The electrophoretic patterns of Fig. 2 and the calculated mobilities in Table II indicate a close similarity among the various samples of semen, and no major characteristic differences are apparent other than some variation in the relative amounts of the constituents. This is especially apparent with some of the minor constituents which, in some of the patterns, are completely absent or obscured by the constituents present in higher concentration.

**Table II**

*Electrophoretic Comparison of Various Bovine Seminal Plasma Protein Samples with Normal Bovine Blood Serum Proteins*

<table>
<thead>
<tr>
<th>Component No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.6</td>
<td>+2.9</td>
<td>−1.3</td>
<td>−2.0</td>
<td>−2.8</td>
<td>−4.0</td>
<td>−4.7</td>
<td>−5.3</td>
<td>−6.5</td>
<td>−7.3</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.5</td>
<td>+3.0</td>
<td>−0.9</td>
<td>−1.7</td>
<td>−2.7</td>
<td>−3.8</td>
<td>−4.6</td>
<td>−5.1</td>
<td>−6.5</td>
<td>−7.2</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.7</td>
<td>+3.2</td>
<td>−1.0</td>
<td>−2.1</td>
<td>−2.8</td>
<td>−3.8</td>
<td>−4.4</td>
<td>−5.2</td>
<td>−6.0</td>
<td>−6.5</td>
<td>−7.1</td>
</tr>
<tr>
<td>D</td>
<td>1.8</td>
<td>+3.0</td>
<td>−1.2</td>
<td>−2.4</td>
<td>−3.3</td>
<td>−4.4</td>
<td>−4.9</td>
<td>−5.3</td>
<td>−5.7</td>
<td>−6.3</td>
<td>−7.0</td>
</tr>
<tr>
<td>E</td>
<td>0.8</td>
<td>+3.1</td>
<td>−1.0</td>
<td>−1.7</td>
<td>−2.7</td>
<td>−3.8</td>
<td>−4.6</td>
<td>−5.1</td>
<td>−5.7</td>
<td>−5.8</td>
<td>−6.2</td>
</tr>
<tr>
<td>F</td>
<td>2.2</td>
<td>+3.0</td>
<td>−1.2</td>
<td>−2.5</td>
<td>−3.2</td>
<td>−3.9</td>
<td>−4.9</td>
<td>−5.3</td>
<td>−5.6</td>
<td>−6.2</td>
<td>−6.0</td>
</tr>
<tr>
<td>G</td>
<td>1.0</td>
<td>+3.1</td>
<td>−1.0</td>
<td>−1.7</td>
<td>−2.7</td>
<td>−3.8</td>
<td>−4.6</td>
<td>−5.1</td>
<td>−5.7</td>
<td>−5.7</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>1.6</td>
<td>+3.1</td>
<td>−1.0</td>
<td>−1.7</td>
<td>−3.0</td>
<td>−4.4</td>
<td>−5.0</td>
<td>−5.4</td>
<td>−5.7</td>
<td>−6.4</td>
<td>−7.0</td>
</tr>
<tr>
<td>I</td>
<td>3.1</td>
<td>+2.9</td>
<td>−1.2</td>
<td>−2.3</td>
<td>−3.2</td>
<td>−4.4</td>
<td>−5.0</td>
<td>−5.6</td>
<td>−6.1</td>
<td>−6.8</td>
<td>−7.5</td>
</tr>
<tr>
<td>J</td>
<td>2.2</td>
<td>+2.9</td>
<td>−1.2</td>
<td>−2.3</td>
<td>−3.2</td>
<td>−4.4</td>
<td>−5.0</td>
<td>−5.6</td>
<td>−6.1</td>
<td>−6.8</td>
<td>−7.5</td>
</tr>
</tbody>
</table>

*See Fig. 2 for identification of conditions and components.
†Mobilities in $1 \times 10^{-3}$ cm$^2$ volt$^{-1}$ sec.$^{-1}$.

A pattern of the normal bovine blood serum proteins obtained under the same electrophoretic conditions is also shown in Fig. 2 ($J$). It is apparent from the patterns that the seminal plasma proteins quantitatively do not resemble those of blood serum. The major components of the seminal plasma (Components 5, 6, and 8, Fig. 2) exhibit mobilities similar to those of the $\alpha_1$, $\alpha_2$, and $\alpha_3$-globulins of blood. Material with the mobility of blood serum albumin is either absent in the seminal plasma or present at a very low concentration (Component 10). Components 2 and 3 in the seminal plasma, which correspond in mobility to the immune $\gamma$, $\gamma'\alpha$, $\gamma'$, T-, and $\beta_2$-globulins named by various investigators (18, 19), may tend to be more concentrated in the samples from the older bulls.

*Further Characterization Methods—Since preliminary studies indicated
that some of the seminal plasma proteins were unstable upon prolonged
dialysis against distilled water, an experiment was conducted to determine
what fractions were precipitated by this treatment. The pooled sample of
seminal plasma (with a few drops of toluene) was dialyzed for 4 days at 4°
against frequent changes of distilled water. Upon centrifugation a clear
supernatant fluid resulted and a white precipitate which was washed twice
by stirring in cold water and dissolved in Veronal buffer. Each of the two
fractions was dialyzed against Veronal buffer and analyzed electrophoretically.
The results (Fig. 3) indicate that, whereas the two patterns are
similar, there are differences in the relative amounts of the major compo-
nents present. Components 10 and 11 (see Figs. 1 and 2) are present only
in the supernatant fluid and hence possess typical albumin characteristics.

A Molisch test for carbohydrate of the two fractions revealed that only
the supernatant solution was positive. This would indicate, since each of
the major electrophoretic components was present in both fractions, that

\[ \text{Precipitate} \]
\[ \text{Supernatant} \]

![Electrophoretic analysis of the precipitable and supernatant fractions produced by exhaustive dialysis of the bovine seminal plasma proteins. Analyses in Veronal buffer, pH 8.58, ionic strength 0.1, for 5400 seconds.](http://www.jbc.org/)

none of them can be classified as typical glycoproteins, a conclusion which
differs from that of Ross et al. (8–10) and Huggins et al. (6, 7) who found a
large glycoprotein content present in human seminal plasma.

**DISCUSSION**

The results of this investigation indicate that the proteins of bovine
seminal plasma are of a heterogeneous nature. However, the similarity
among samples collected from different animals is close.

The nitrogen content of 14.4 per cent for the total protein of plasma is
low compared to the values reported for most other proteins. Since agree-
ment was obtained between two exhaustively dialyzed samples, one of
which had been extracted with ether, there is apparently a negligible
amount of ether-extractable materials present in the dialyzed seminal
plasma. Also since the Molisch test signified a low carbohydrate content,
it would appear that the low nitrogen content is not due to a large lipo-
or glycoprotein content, and hence the proteins themselves must be low
in the basic amino acids. It is interesting to speculate that the seminal
plasma proteins may be formed under conditions in which most of the basic
amino acids are taken up preferentially by the sperm cells.
Electrophoretic evidence shows that the majority of the bovine seminal plasma proteins possess mobilities similar to the α-globulins of blood. These proteins are known to be largely lipo- or glycoproteins in nature, of which the former exist as somewhat unstable complexes (19). Freezing, thawing, heating, extraction, and other physical manipulations are known easily to unstabilize these proteins irreversibly (19). Since an extremely low carbohydrate and lipide content and stability to physical treatment were indicated, it would appear that the majority of the seminal proteins are not the same as the α-globulins of blood. Further evidence to support this conclusion will be reported in a subsequent paper on some ultracentrifugal and immunological studies of the bovine seminal plasma proteins.

**SUMMARY**

Characterization of the bovine seminal plasma has indicated that approximately 90 per cent of the total nitrogen present is protein in nature and non-dialyzable; 76 per cent of these proteins were heat-coagulable. Seminal plasma proteins, after exhaustive dialysis against water, gave an extremely low test for carbohydrate and fat and contained 14.4 per cent nitrogen (fat- and moisture-free basis).

Electrophoretic studies indicated the presence of at least eleven components. However, three of these accounted for the majority of the proteins present. A great similarity was evident in the electrophoretic patterns of the seminal plasma proteins obtained from bulls of various breeds and ages. Comparative electrophoretic analyses of the bovine blood serum and the seminal plasma proteins indicated that there was little quantitative relationship between the two systems. The majority of the protein constituents of seminal plasma exhibited electrophoretic mobilities similar to those of the α-globulins of blood. Chemical evidence, however, indicated that glyco- or lipoproteins were either absent or present in low concentration.

**BIBLIOGRAPHY**

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