HEXOSAMINE-CONTAINING GLYCOPROTEINS OF NORMAL BOVINE SYNOVIAL FLUID*

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Synovial fluid is usually considered to be a modified extracellular fluid or blood dialysate (1, 2). It is now well known that all of the electrophoretically classified groups of proteins found in serum are also, at least qualitatively, present in synovial fluid (3-8). This does not mean, however, that all proteins can pass the capillary membranes, because cholesterol and fats, usually associated with lipoproteins, are absent from the normal fluid (1, 2). The proportion of albumin is greater in human and bovine synovial fluids than in the respective serum, suggesting that a slight generalized permeability (or perhaps selective permeability) exists for molecules of molecular weights of about 65,000 or less. On this account, glycoproteins of similar or smaller molecular weight might also be present in synovial fluid to a greater degree than in serum. It is the purpose of this report to demonstrate a generalized enrichment of α- and β-hexosamine-containing glycoproteins1 in synovial fluid.

Bovine fluids and sera were used because of the ease with which these materials are obtained; sufficient fluid is generally obtainable from human subjects only as traumatic effusions. Schmid et al. have shown that such effusions contain many of the proteins common to serum (9, 10). Unfortunately trauma is also accompanied by an increase in permeability (2); hence, data obtained with such fluids do not strictly describe the normal composition. One might also object that bovine synovial fluid obtained at a slaughterhouse is not quite normal, as the animals may have stood for long periods of time in transit. Nevertheless, it is felt that carefully selected bovine fluids will present a relatively normal picture of the glycoproteins present.

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1 Glycoprotein, in this paper, is used in connection with any hexosamine-containing protein.
GLYCOPROTEINS OF BOVINE SYNOVIAL FLUID

EXPERIMENTAL

Synovial fluids were aspirated from both astragalotibial joints of 3 to 5 year-old beef heifers immediately after slaughter. A sample of blood from the same animal was obtained and allowed to clot. Sera and the combined fluids from both joints of a single cow were centrifuged and used on the same day.

Before electrophoresis, a 10 ml. sample of synovial fluid was treated with 0.5 ml. of hyaluronidase (75 turbidity reducing units) to reduce the viscosity. Preliminary tests showed that the viscosity was minimal after letting this mixture stand at 5° for 48 hours. The synovial fluid was then concentrated by dialysis for 16 hours against a 20 per cent (by weight) solution of dextran at 5°.

Both the serum, 3 ml. samples, and the concentrated synovial fluids were separated by zone electrophoresis on starch by the procedure previously outlined (11, 12). The Verona buffer had an ionic strength of 0.075 and a pH of 9.1. The protein content of the eluates was measured by the method of Lowry et al. (13), and determinations of hexosamine were made by the isoamyl alcohol extraction procedure (14). A biuret method was used for the total proteins of serum and synovial fluid (15).

Results

The protein and hexosamine contents of each eluate were plotted as per cent of the total versus the distance migrated from the origin as shown in Fig. 1. The migration was expressed as the fraction, Ralumin, which is the distance migrated divided by the distance of the albumin peak from the origin. Fig. 1 is a typical protein and hexosamine pattern for synovial fluid and is qualitatively the same as the patterns obtained with serum. To evaluate the protein pattern the areas bounded by the albumin, α-, β-, and γ-peaks were measured with a planimeter. For glycoproteins only the α-, β-, and γ-areas were used, because no significant amount of hexosamine appears in the albumin region (cf. Fig. 1). From the average relative amounts of each fraction (Fig. 2) it is apparent that the albumin content of synovial fluid is greater than that of serum (50.0 per cent versus 38.6 per cent), α is lower (12.1 per cent versus 14.2 per cent), β values are the same (12.3 per cent versus 12.6 per cent), and the γ proteins are lower in fluid than in serum (25.5 per cent versus 34.8 per cent). The data for serum

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2 Synovial fluid and sera were obtained through the courtesy of Standard Beef, Inc., Detroit, Michigan.

3 We are grateful to the Wyeth Laboratories, Inc., Philadelphia, Pennsylvania, for generous contributions of Wydase.

4 Gratitude is expressed to the Commercial Solvents Corporation, Terre Haute, Indiana, for generous supplies of dextran.
alone are the same as the results obtained in preliminary studies by paper electrophoresis and are comparable with those reported by others (16–19). Also the differences in albumin, α-, β-, and γ-zones between serum and synovial fluid are the same as those previously reported (6, 8). The average relative amounts of hexosamine (Fig. 3) show that the α component of synovial fluid is slightly greater than that of serum (59.6 per cent versus 56.7 per cent), the β component is higher (21.3 per cent versus 16.7 per cent), and the γ component is lower (19.1 per cent versus 26.6 per cent) in fluid than in serum.

The relative amounts of protein and hexosamine were then used to calculate the concentrations of hexosamine in the total protein of the α-, β-, and γ-peaks (Table I). The differences in hexosamine concentration between fluid and serum are also given.

Synovial fluid taken directly from the joint is too viscous for a clear electrophoretic separation on a starch medium. The need for a preliminary

![Fig. 1. Typical protein and glycoprotein patterns for normal bovine synovial fluid. Patterns for bovine serum differed only in proportions of the zones. Peaks are named in accordance with convention; i.e., albumin at $R_{ALB} = 1.0$ followed by α, β, and finally γ at $R_{ALB}$ of 0.2 to −0.3. The protein and hexosamine values are expressed as per cent of total present in all the eluates.](http://www.jbc.org/)

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depolymerization of the hyaluronic acid by hyaluronidase introduced possible alterations of protein mobilities and proportions of the zones. Also, the products of depolymerization might have combined with proteins and changed the normal quantities of hexosamine in each zone. A paper electrophoresis technique was used in preliminary studies to test these possibilities. Fresh bovine serum was diluted to 1 per cent protein, treated with hyaluronidase, and concentrated either by dialysis against dextran or by pressure dialysis. After electrophoretic separation, the proteins were stained with bromophenol blue. The albumin, α-, β-, and γ-zones were eluted with 2 per cent sodium carbonate in 50 per cent methanol, and the optical densities of the eluates were measured at 595 nm after 30 minutes (20). The proportions of albumin, α-, β-, and γ-zones were the same as those with the untreated serum. Thus neither hyaluronidase nor the concentrating process had any undesirable distorting effect upon the protein pattern.

*Paper electrophoresis was performed with the Spinco apparatus on Whatman No. 3MM paper and with barbital buffer, pH 9, of 0.075 μ.*

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**Fig. 2.** Comparison of average proportions of electrophoretically separated groups of proteins from bovine serum and synovial fluid.
The effects of hyaluronidase and the products of hyaluronic acid depolymerization on the proportions of glycoproteins were determined by separating electrophoretically the proteins of (1) a diluted sample of serum and (2) a diluted sample of serum containing purified hyaluronic acid (21). Both samples were treated with hyaluronidase and concentrated. The paper electropherograms were cut into α-, β-, and γ-zones, and each paper section was boiled with 3 n HCl. The hexosamine contents of the hydrolysates were then measured (14). In this experiment corrections were made for a little color produced by the hydrolysis of paper alone. Again, no significant differences in proportions of hexosamine in the α-, β-, and γ-zones were observed between the treated samples and the original serum, thereby demonstrating that neither the enzyme nor the products of the depolymerization of hyaluronic acid had an observable effect upon the electrophoretic pattern.

![Electrophoretically separated hexosamine-containing glycoproteins of bovine serum compared with those of synovial fluid.](image)

Fig. 3. Electrophoretically separated hexosamine-containing glycoproteins of bovine serum compared with those of synovial fluid.
DISCUSSION

Some of the \( \alpha \)-globulins of serum (human) have a relatively low molecular weight and a considerable content of hexosamine \( (22, 23) \). Orosomucoid, which constitutes about 10 per cent of the total glycoproteins, has a molecular weight of 44,100 and 11.5 per cent hexosamine \( (24) \). Other low molecular weight \( \alpha \)-glycoproteins have been observed to have a hexosamine content of 3.5 to 4 per cent \( (25) \). Such proteins might preferentially pass through capillary membranes and accumulate in the synovial fluid. An increase in the proportion of hexosamine-containing \( \alpha \)-glycoproteins in synovial fluid is demonstrated by Fig. 3. At the same time there is a reduction in the proportion of \( \alpha \)-globulins (Fig. 2). These two effects result in the higher percentage of hexosamine in the \( \alpha \)-globulins of synovial fluid as compared with serum (Table I).

Little information is available concerning individual glycoproteins migrating as \( \beta \)-globulins. Fig. 3 shows that some hexosamine-rich \( \beta \)-glycoproteins do accumulate in the synovial fluid, while, on the other hand, the total \( \beta \)-globulins do not show a significant difference between serum and fluid. Thus there is a higher percentage of hexosamine in the synovial fluid fraction than in serum (Table I). The increased concentrations

TABLE I

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Serum</th>
<th>Synovial fluid</th>
<th>Difference*</th>
</tr>
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<tr>
<td></td>
<td>Total protein</td>
<td>( \alpha )</td>
<td>( \beta )</td>
</tr>
<tr>
<td>1</td>
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<td>4.1</td>
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<td>7.0</td>
<td>3.8</td>
<td>1.4</td>
</tr>
<tr>
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<td>8.2</td>
<td>3.3</td>
<td>1.0</td>
</tr>
<tr>
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<td>5.4</td>
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<tr>
<td>14</td>
<td>6.8</td>
<td>4.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Average...</td>
<td>7.3</td>
<td>3.8</td>
<td>1.3</td>
</tr>
<tr>
<td>S.d.</td>
<td>( \pm 1.2 )</td>
<td>( \pm 0.4 )</td>
<td>( \pm 0.2 )</td>
</tr>
<tr>
<td>( t ) ‡</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Synovial fluid minus serum.
† Standard deviation.
‡ Student's \( t \) test.
§ Significant at 1 per cent level.

The values given for \( \alpha \)-, \( \beta \)-, and \( \gamma \)-peaks are the hexosamine concentrations in per cent of total proteins in that peak. The values for total protein are given in gm. per 100 ml.
of hexosamine in the β- as well as in the α-peaks are significant at the 1 per cent level (Table I). A complication which cannot be ruled out at present is the possibility that some of these hexosamine-containing globulins are directly of tissue origin.

The concentrations of hexosamine in the proteins of the γ-peaks were the same for serum and synovial fluid (Table I). This suggests that there is no preferential permeability for these glycoproteins. Available evidence indicates that γ-globulins are predominantly proteins of high molecular weight (26).

The present data demonstrate an enrichment of α- and β-globulins with hexosamine-containing glycoproteins in synovial fluid as compared with the serum. No comparisons of albumin have been made because it is felt that this fraction does not have a significant content of hexosamine (12, 22, 27) (cf. Fig. 1).

**SUMMARY**

Proteins of bovine sera and synovial fluids were separated by zone electrophoresis on starch. Analyses for protein and hexosamine were made on the consecutive eluates. The average relative distributions of hexosamine in the electrophoretically separated zones were 59.6 per cent and 56.7 per cent in the α-peaks, 21.3 per cent and 16.7 per cent in the β-peaks, and 19.1 per cent and 26.6 per cent in the γ-peaks of synovial fluid and serum, respectively. The concentration of hexosamine in the total protein of each zone shows an enrichment of hexosamine-containing glycoproteins in the α- and β-peaks of synovial fluid and no change in the γ-region.

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