# Paper Electrophoresis of Human Synovial Fluid.*† (24108)

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Paper electrophoresis of human synovial fluid was performed to determine electrophoretic distribution§ of protein fractions and to separate hyaluronate from proteins. Studies were done on synovial fluid obtained from normal subjects and from a few patients with osteoarthritis.

*Methods.* Synovial fluid was obtained from knee joints of post-mortem subjects(1) and from patients with osteoarthritis. One part (by weight) of sodium diethyl barbiturate buffer (pH 8.6, $\Gamma/2 = .075$) was mixed with 3 parts (by weight) of synovial fluid. This buffer contained 75 turbidity-reducing units (TRU) of testicular hyaluronidase (Wyeth)/0.1 g. Usually 0.1 g of buffer (containing 75 TRU of hyaluronidase) and 0.3 g of synovial fluid were used. This mixture was then incubated 4 hours at 37°C. Paper electrophoresis was performed in Spinco Model R apparatus with eight 3 cm Whatman 3MM papers and sodium diethyl barbiturate buffer (pH 8.6, $\Gamma/2 = .075$) at a voltage gradient of 3 volts/cm for 16 hours.

*Standards.* The hyaluronate standard was a sample of partially purified hyaluronate prepared from synovial fluid from a subject with osteoarthritis, and contained 23% hexosamine and 7% nitrogen. The normal serum standard was prepared from pooled normal sera. *Stains.* a) Bromphenol blue. An amount of synovial fluid or serum containing approximately 350 $\gamma$ of protein was applied. The strips were stained with bromphenol blue as described by Block, Durrum, and Zweig(2), using staining time of 6 hours. Spinco Analytrol was used to determine amount of dye taken up by each protein fraction. b) Periodic acid Schiff (PAS). Synovial fluid or serum containing approximately 1200 $\gamma$ of protein was applied. Staining was performed according to Köiw and Grönwall(3). c) Mucicarmine and toluidine blue. Synovial fluid containing 30 $\gamma$ of hyaluronate hexosamine was applied. The staining of hyaluronate with mucicarmine and toluidine blue has been described elsewhere(4). Prior to staining with toluidine blue, the strips were heat-fixed at 100°C for 30 minutes and were not fixed in alcohol-formaldehyde solution as previously reported. *Analytical methods.* Protein concentration of synovial fluid was determined by biuret method(5) using normal serum as standard. The protein nitrogen of serum was determined by micro-Kjeldahl procedure(6). Hyaluronate was measured as hexosamine(1).

*Results.* 1) *Normal synovial fluid.* After digestion with hyaluronidase the migration by paper electrophoresis of proteins in normal synovial fluid was identical to migration of protein fractions of serum. Percentage of albumin was higher, and percentage of $alpha_2$ and of gamma globulins was lower in normal synovial fluid than in normal serum (Table I).

$Alpha_2$ globulin zone of normal synovial fluid stained much less intensely with PAS than the $alpha_1$ globulin zone. This is a reversal of the staining pattern of alpha globulins of normal serum with PAS(7) and may be due in part to decreased concentration of $alpha_2$ globulins in normal synovial fluid.

Hyaluronate of hyaluronidase-treated normal synovial fluid migrated faster than albumin, had a mobility identical to the similarly-treated hyaluronate standard, stained metachromatically with toluidine blue (Fig. 1), did not stain with bromphenol blue, was PAS

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§ By electrophoretic distribution of protein fractions of synovial fluid is meant amount of protein in each fraction separated by paper electrophoresis expressed as % of total protein of fluid.

TABLE I. Electrophoretic Distribution of Proteins of Normal and Osteoarthritic Synovial Fluid.

| | Protein conc., g/100 g | Distribution of proteins (%) | | | | |
|---|---|---|---|---|---|---|
| | | Albumin | Globulins | | | |
| | | | Alpha$_1$ | Alpha$_2$ | Beta | Gamma |
| Pooled normal sera | 6.8 | 52 | 5 | 11 | 13 | 19 |
| *Normal synovial fluid* | | | | | | |
| Age, 1 yr | | 68 | 6 | 6 | 12 | 8 |
| 2 | | 71 | 5 | 4 | 11 | 9 |
| 23 | 2.7 | 81 | 3 | 3 | 7 | 6 |
| 25 | | 70 | 3 | 3 | 14 | 10 |
| 29 | 1.6 | 80 | 3 | 3 | 6 | 8 |
| 29 | | 79 | 5 | 2 | 7 | 7 |
| 42 | 2.5 | 67 | 7 | 5 | 10 | 11 |
| 57 | 2.6 | 76 | 4 | 3 | 10 | 7 |
| 58 | 1.8 | 69 | 4 | 5 | 11 | 11 |
| 63 | 2.5 | 74 | 4 | 3 | 8 | 11 |
| 71 | | 68 | 5 | 4 | 11 | 12 |
| 76 | 1.2 | 65 | 6 | 5 | 13 | 11 |
| *Osteoarthritic synovial fluid* | | | | | | |
| 54 | | 53 | 6 | 6 | 15 | 20 |
| 57 | 3.5 | 60 | 4 | 3 | 13 | 20 |
| 59 | 2.7 | 56 | 8 | 7 | 12 | 17 |
| 67 | 2.4 | 55 | 6 | 6 | 17 | 16 |
| 69 | 2.8 | 58 | 9 | 8 | 12 | 23 |

negative, and stained with mucicarmine.

2) *Osteoarthritic synovial fluid.*‖ After digestion with hyaluronidase, migration by paper electrophoresis of proteins and hyaluronate of osteoarthritic synovial fluid was identical to the migration of similar components of normal fluid. Percentage of albumin was lower and percentage of gamma globulins was higher in osteoarthritic fluid than in normal fluid (Table I). Percentage of albumin was slightly higher and percentage of alpha$_2$ globulins lower than in normal serum (Table I). Electrophoretic distribution of proteins in osteoarthritic serum was similar to normal serum.

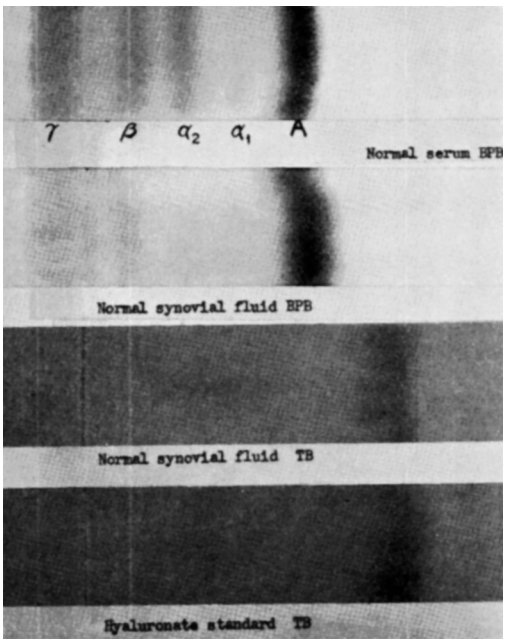


FIG. 1. Paper electrophoresis of hyaluronidase-treated normal synovial fluid, hyaluronidase-treated hyaluronate standard, and normal serum stained with bromphenol blue (BPB) and toluidine blue (TB).

Staining reactions of components of osteoarthritic fluid with bromphenol blue, PAS, mucicarmine, and toluidine blue were identical to those of normal fluid.

*Discussion.* The use of paper electrophoresis to determine distribution of proteins of normal synovial fluid has overcome difficulties encountered by workers using free electrophoresis. In a recent study(8) of normal synovial fluid by free electrophoresis, the concentration of globulins was too low to permit electrophoretic distribution of proteins in

‖ Synovial fluid obtained from osteoarthritic joints will be briefly termed osteoarthritic synovial fluid.

these fractions to be determined. The small volume of synovial fluid in normal joints has led some investigators(9) to use fluid from traumatized joints as a basis with which to compare changes in electrophoretic distribution of synovial fluid proteins in diseases of the joints, but an increased volume of fluid and a higher concentration of protein set traumatic synovial fluid apart from normal fluid. The technic of paper electrophoresis reported here has permitted analysis of very small amounts of synovial fluid from normal subjects ranging in age from 1 to 76 years and has revealed marked differences in electrophoretic distribution of protein fractions of normal synovial fluid and normal serum. Differences in distribution of synovial fluid proteins with age were not found.

Electrophoretic distribution of proteins in synovial fluid of a few patients with osteoarthritis was determined. Distribution of protein in these fluids differed from that found in synovial fluid obtained from normal subjects of a similar or older age than patients with osteoarthritis. This suggests that the underlying process in osteoarthritis is not simply one of "accelerated normal aging."

Permeability(10) of normal synovialis may determine which plasma proteins gain access to the synovial fluid, but factors other than permeability must operate to keep the albumin concentration (% albumin x protein concentration—Table I) of normal fluid at a level one half that of plasma.

Staining reactions of hyaluronate separated from proteins of hyaluronidase-treated synovial fluid by paper electrophoresis were of interest. Failure of bromphenol blue to stain hyaluronate indicated that the protein content of hyaluronate was low. Hyaluronate did not give a positive PAS reaction. This finding agrees with reports(11,12) that hyaluronate fails to react with PAS when it is free of protein-bound components which stain with PAS. Hyaluronate stained metachromatically with toluidine blue although partially depolymerized by hyaluronidase. Depolymerization of synovial fluid hyaluronate was indicated by the fact that the relative viscosity of normal synovial fluid, measured in an Ostwald viscometer at 37°C, fell from a value exceeding 500 to 1.5 within 4 hours after the addition of testicular hyaluronidase in the barbital buffer (pH 8.6). Since the pH of the synovial fluid was not lowered to 5 prior to addition of hyaluronidase, depolymerization of hyaluronate was not sufficient to render it dialyzable(1). Persistence of metachromasia of depolymerized hyaluronate has not been previously noted, and is of interest since depolymerization has been thought to destroy the chromatropic property of hyaluronate(13).

*Summary.* After treatment of human synovial fluid with hyaluronidase, the hyaluronate and proteins can be separated by paper electrophoresis. The electrophoretic distribution of the protein fractions of normal synovial fluid differs from that of the protein fractions of both normal serum and osteoarthritic synovial fluid.

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