Hyaluronic Acid in Human Synovial Effusions; A Sensitive Indicator of Altered Connective Tissue Cell Function During Inflammation

By C. WILLIAM CASTOR, ROBERT K. PRINCE AND MARGARET J. HAZELTON

STUDIES OF HUMAN JOINT FLUID have sought to relate hyaluronic acid concentration and parameters of molecular weight to the various rheumatic diseases associated with synovial effusions. It is difficult to compare data in published studies largely because of: (1) a paucity of observations on individual normal joint fluids and (2) a lack of uniform methodology in measurement of hyaluronic acid and performance of physical studies.

In one of the most comprehensive studies, Sundblad examined 2 normal fluids and found the hyaluronic acid concentration to be 2.97 mg./ml. and the mean intrinsic viscosity (a parameter molecular weight) to be 39.3.[1] His study indicated that rheumatoid, degenerative, and traumatic arthritis all led to a significant decrease in concentration of hyaluronic acid. Intrinsic viscosity was decreased in rheumatoid arthritis, degenerative joint disease, and infectious arthritis but not in traumatic arthritis. These studies suggested that neither the size and duration of effusion, nor systemic parameters such as the erythrocyte sedimentation rate correlated with the degree of mucopolysaccharide abnormality. Hamerman and Schuster provided the first evidence suggesting that normal values for the hyaluronic acid (HA) content of joint fluids might vary with age.[2] Their study showed that persons under age 40 had a mean HA concentration in synovial fluid of 3.6 mg./ml., less being found in persons 46 to 85 years of age. Their study of fluids from patients with rheumatoid arthritis confirmed the low HA concentration and questioned the importance of minor differences in viscosity.[3] Bollet measured the HA concentration and viscous properties of postmortem and pathologic fluids and obtained data indicating that intrinsic viscosity was decreased in traumatic arthritis, osteoarthritis, acute gout, and rheumatic fever as well as in rheumatoid arthritis.[4] Oral adrenocorticosteroid therapy had little affect on intrinsic viscosity in seven of eight cases. Stafford et al. showed that HA concentration was depressed in effusions from patients with systemic lupus erythematosus, gout, osteoarthritis, rheumatoid arthritis, traumatic arthritis, and ankylosing spondylitis.[5] In most varieties of inflammatory synovitis they recorded a depression of intrinsic viscosity (using a different buffer system than other workers), when compared to normal persons examined by the same procedure. The mean HA concentration in fluids from eight normal volunteers was 4.1 mg./ml. Recently Seppälä demonstrated that favorable changes in the HA of rheumatoid joint effusions after treatment with intra-articular

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steroids were linked to objective evidence of a good clinical response to the injected steroid.[6]

In the present series of investigations we measured HA concentration and intrinsic viscosity in normal joint fluid from individuals of varying age. It was the purpose of this survey to apply the same technical methods to a variety of rheumatic afflictions and to compare the findings with normal values in corresponding age ranges. In many instances joint fluid white cell counts were also available. In another phase of this study two volunteers with rheumatoid arthritis were studied intensively with respect to the characteristics of re-forming synovial effusions. The qualitative and quantitative aspects of HA formation were examined at 12 hour intervals and the effects of intra-articular hydrocortisone were assessed.

METHODS

Joint fluid was aspirated from six normal ambulatory medical students and hospital employees. A group of ten ambulatory patients with no history of findings of articular disease (age range: 60 to 82 years) was studied to provide information concerning older persons. One per cent xylocaine was used to infiltrate the skin and capsular tissues.

Pathologic fluids were obtained at the time of either diagnostic or therapeutic arthrocentesis. Most fluids were processed immediately following aspiration, and the occasional sample that was not processed for 24 hours was stored at 4 C. until viscometry was carried out. Normal fluids were diluted 1:50 or 1:100 with 0.05 M phosphate, pH 7.0 buffer which was 0.15 M with respect to sodium chloride. Pathologic fluids were usually diluted 1:25 with the same buffer. Prior to viscometric or chemical analysis either the original joint fluid or the dilution was centrifuged at 17,000 × g. for 10 minutes to remove particulate matter. The Ostwald pipettes used for viscometry had a 3.0 ml. upper bulb capacity and buffer flow times of 60 to 70 seconds. Viscometers were loaded with 4.0 ml. of diluted joint fluid and brought to 37 C. before making multiple measurements of the efflux time of the polymerized specimens. The flow time of the depolymerized specimen was determined following addition of 0.05 ml. of a testicular hyaluronidase solution in the same buffer (1.0 mg. or 300 turbidity reducing units*). A 5 minute incubation permitted maximum viscosity reduction of all samples.[7]

Chemical measurement of the HA content of joint fluid specimens was carried out on the depolymerized specimen removed from the viscometer, using a procedure similar to that of Decker et al. [8] For this determination an aliquot of depolymerized joint fluid dilution was mixed with an equal volume of 20 per cent trichloroacetic acid in water. Acidified joint fluid dilution was brought to a boil in a waterbath over a gas flame and then centrifuged to remove the protein precipitate. The supernatant fluid was used for measurement of HA, using a borate modification of the Dische carbazole procedure. [9] At the dilutions employed hyaluronidase digestion precludes appreciable co-precipitation of HA with protein during the acidification and heat coagulation steps. The amount (micromoles) of uronic acid in samples was estimated from a standard curve employing glucuronolactone, and the conversion of uronic acid to HA assumed a disaccharide molecular weight of 400. Calculations of intrinsic viscosity were made with the empiric formula developed by Sundblad:

$$[\eta] = \frac{\eta sp}{c(1 + 0.18 \times \eta sp)}.$$

Intrinsic viscosity determinations made in the presence of physiologic pH and electrolyte concentration are related to molecular weight by the following expression:

$$[\eta] = 0.036 \times M^{0.78}.$$

Laurent demonstrated that this relation was valid for HA preparations with molecular weights of 7.7×10^4 - 1.7×10^6 using light scattering as the reference parameter for molecular weight.[10] A recent report indicates that the expression is valid for high polymer hyaluronate, (Ox HA, MW = 15×10^6).[11]

Protein was measured either by the method of Oyama and Eagle[12] or by a spectrophotometric method. White cell counts on joint fluid were made using a standard hemocytometer and employing 0.12 N hydrochloric acid as diluent. In selected samples sodium and potassium were measured with an autoanalyzer flame photometer, and calcium was determined by the Clark-Collip method.[13] Plasma proteins were resolved in both

*Worthington Biochemical Corp., Freehold, N. J.

Table 1.—*Joint Fluid Characteristics in Absence of Known Rheumatic Disease*

Source of Joint Fluid	Subject Ages	No. of Subjects	Hyaluronic Acid Concentration (mg./ml.) Mean ± S.E.M.	Intrinsic Viscosity (dl./Gm.) Mean ± S.E.M.	Molecular Weight, $\times 10^6$	Authors
Normal knees	—	2	2.97	39.3	2.85*	Sundblad, 1953
Normal joints	—	8	3.21 ± 0.13	—	—	Decker, 1959
Normal joints	—	8	4.10 ± 0.10	—	—	Stafford et al., 1964
Postmortem joints	—	8	1.72 ± 0.40†	—	—	Bollet, 1956
Postmortem joints	—	13	2.40 ± 0.09	—	—	Stafford et al., 1964
Postmortem joints	9–37	11	4.06 ± 0.22†	—	—	Hamerman & Schuster, 1958
Postmortem joints	46–53	6	2.88 ± 0.18†	—	—	Hamerman & Schuster, 1958
Postmortem joints	62–85	9	2.25 ± 0.15†	—	—	Hamerman & Schuster, 1958
Normal knees	21–42	6	3.74 ± 0.20	36.9 ± 0.9	2.65	Castor & Prince, 1964
Normal knees	60–82	10	2.00 ± 0.25	38.0 ± 1.4	2.75	This study
Amputated knee	44	1	1.64	40.4	2.95	This study
Amputated knee	65	1	2.14	51.1	4.00	This study

*Except where specifically noted, molecular weight values in these tables are derived from intrinsic viscosity measurements.

†These values were computed from published data.

plasma and joint fluid specimens employing a Spinco paper electrophoresis apparatus and a Spinco analytrol. Joint fluid specimens were preincubated with hyaluronidase before electrophoresis.

Results

Characteristics of Normal Joint Fluid

Data from previous studies, in some cases recalculated from the author's published data, are presented in Table 1. The omission of intrinsic viscosity data reflects either the omission of viscosity measurements by the author or technical differences precluding their consideration in relation to the present study. These differences include dilute electrolyte concentrations, unusual buffer systems, and measurement of relative viscosity in concentrated HA solutions. As is apparent from the table very few samples of normal human joint fluid have been examined individually for HA concentration and intrinsic viscosity. The study of Hamerman and Schuster on postmortem fluids suggested that the HA concentration in older individuals was approximately one-half that of the younger donors. Stafford et al. report values around 4.0 mg./ml. for normal joints and make the point that postmortem fluids show a significantly decreased HA concentration. This latter study does not record the ages of the donors of normal and postmortem joint fluid, leaving open the possibility that the difference seen may have been related to age rather than to the vitality of the donor. The conditions established by Sundblad resulted in intrinsic viscosity measurements averaging 39.3 in two normal subjects. Intrinsic viscosity determined in this manner was subsequently shown to be a measure of molecular weight.[10]

Measurements of HA concentration made in our laboratory on joint fluid from six young normal subjects revealed a mean value agreeing well with the majority of the other published values.[14] The intrinsic viscosity measurements were similar to

Table 2.—*Joint Fluid Characteristics in More Common Rheumatic Afflictions*

Diagnosis	Age*	No. of Patients	Hyaluronic Acid Concentration (mg./ml.) Mean ± S.E.M.	P†	Intrinsic Viscosity (dl./Gm.) Mean ± S.E.M.	P	Molecular Weight × 10^6
Degenerative joint disease	57	6	1.90 ± 0.17	N.S.	44.7 ± 7.71	N.S.	3.40
Traumatic synovitis	25	10	0.72 ± 0.11	<0.01	27.8 ± 2.87	<0.02	1.85
Rheumatoid spondylitis	41	4	1.18 ± 0.18	<0.01	28.6 ± 3.50	<0.01	1.90
Ulcerative colitis	21	3	0.92 ± 0.14	<0.01	19.3 ± 4.63	<0.01	1.15
Reiter's syndrome	36	3	0.86 ± 0.19	<0.01	22.6 ± 3.53	<0.01	1.41

*Mean age of group.
†Derived from Student-Fisher + distribution.

those reported by Sundblad. More recent study of joint fluid aspirated from individuals over 60 years of age showed intrinsic viscosity values similar to that of the younger individuals. The data relevant to HA concentration clearly confirm the impression of Hamerman and Schuster that the HA concentration of the older individual is lower. The volume of fluid aspirated from the joints of younger individuals varied from 0.2 ml. to as high as 1.5 ml. on two occasions. Fluid was somewhat more difficult to remove from the older population, 0.1 to 0.5 ml. being the more common volumes found, although as much as 1.0 to 2.0 ml. was removed occasionally.

Characteristics of Pathologic Joint Fluid

As noted in Table 2, the mean age of patients with degenerative joint disease in this study was 57 years.* The HA concentration in these fluids was not significantly different than in normal fluids from persons of similar age. Furthermore, the mean of intrinsic viscosity determinations in this group of individuals was not significantly different from normal. These findings are contrary to those reported by Sundblad and by Stafford et al., but are in substantial agreement with the data reported by Decker[8] and Bollet.[4]

Traumatic synovitis was encountered in ten individuals, mostly young athletes who had recently sustained knee injuries in basketball or football. There was one bloody effusion, the remainder being clear fluids with white cell counts ranging from 350 to 5,000. The HA concentration in the traumatic effusions was significantly depressed when compared with the mean value seen in the younger age group. The intrinsic viscosity was similarly markedly and significantly depressed. The finding of the reduced HA concentration and intrinsic viscosity in traumatic synovitis is in substantial agreement with Bollet and with Stafford et al. Patients with rheumatoid spondylitis, arthritis associated with ulcerative colitis, and Reiter's syndrome also demonstrated marked reduction in HA concentration and intrinsic viscosity. In each disease group the differences were significant in spite of the small population sampled. It is of interest that the patients with ulcerative colitis and Reiter's syndrome exhibited some of the lowest intrinsic viscosity values which we observed. One patient with ulcerative colitis had effusion fluid with an intrinsic viscosity of 11.0 (MW = 0.55 × 10^6), and values of 18.0 and 19.0 were commonly seen in these two diseases. The one patient with rheumatoid spondylitis and peripheral joint involvement examined by Sundblad had essentially normal values, while the report by Stafford et

*Patients with degenerative joint disease were older persons with a typical pattern of articular pain and swelling, without constitutional symptoms, with normal or low joint fluid white cell counts, without laboratory evidence suggesting inflammatory synovitis, and with compatible x-rays when these were available.

Table 3.—*Joint Fluid Characteristics in Rheumatoid Arthritis*

Diagnosis	Age	No. of Patients	Hyaluronic Acid Concentration (mg./ml.) Mean ± S.E.M.	P	Intrinsic Viscosity (dl./Gm.) Mean ± S.E.M.	P	Molecular Weight × 10^6	Authors
Rheumatoid arthritis:	—	40	1.52	—	26.3 ± 1.2	—	1.73	Sundblad, 1953
	—	26	1.15	—	—	—	—	Decker, 1959
	—	23	0.96	—	Decreased	—	—	Bollet, 1956
	—	22	0.89	—	Similar or slightly decreased	—	—	Hamerman, 1958
	—	21	0.70		Decreased	—	—	Stafford et al., 1964
	—	48	0.99 ± .07	—	36.8 ± 1.13	—	1.90*	Seppälä, 1964
	18–43	6	1.13 ± 0.10	<0.01	27.3 ± 3.34	<0.01	1.80	Present study
	46–60	16	1.27 ± 0.14	<0.01	31.6 ± 1.70	<0.01	2.15	Present study
	61–75	10	1.31 ± 0.14	<0.01	30.1 ± 2.08	<0.01	2.03	Present study

*This value was calculated from Ogston's equation, which includes the sedimentation coefficient as well as intrinsic viscosity measurements.[15]

Table 4.—*Joint Fluid Characteristics in Effusions Seen with Other Diseases*

Diagnosis	Age	Joint Fluid WBC/mm.^{3}	HA Conc. (mg./ml.)	Intrinsic Viscosity (dl./Gm.)	Molecular Weight × 10^6
Polymyositis	52	7,500	1.37	19.9	1.22
Scleroderma	53	Clear	1.56	36.8	2.64
Polyarteritis	33	2,450	1.54	30.2	2.05
Sporotrichosis	65	—	1.22	35.5	2.51
Relapsing polychondritis	39	Ankle*	0.99	37.0	2.65
Relapsing polychondritis		Knee, 7,650	1.40	21.6	1.33
Pseudogout	46	1,150	0.87	17.3	1.00
Hodgkins disease	32	19,600	1.73	20.6	1.25

*Fluid from the ankle was purulent, and a biopsy showed chondritis without evidence of synovitis.

al. includes findings similar to our own. Decker's report includes one example of Reiter's syndrome in which the HA concentration was reported markedly decreased. We found no reports of HA measurements in patients with ulcerative colitis.

Joint fluid HA data in rheumatoid arthritis are presented in Table 3 and demonstrate that depression of HA concentration and intrinsic viscosity was found irrespective of patient age. These findings are in agreement with other studies in which comparable methods were employed.

Individual patients with effusions related to less common clinical problems were studied in several instances, and the relevant data are presented in Table 4. The patient with scleroderma, however, had sclerosis of the skin over the aspirated knee without clinical evidence of effusion.

Joint Fluid WBC Count and Intrinsic Viscosity of Hyaluronate

Examination of joint fluids from patients with degenerative joint disease and traumatic synovitis showed white blood cell counts ranging from 250/ml.^{3} to 5,000/ml.^{3} In the small number of fluids in which both sets of measurements were available there was no consistent relationship between the two sets of variables. In one patient a white blood cell count of 5,000 was associated with an intrinsic viscosity of 37.6, while another fluid with a similar intrinsic viscosity had a white cell count of 400.

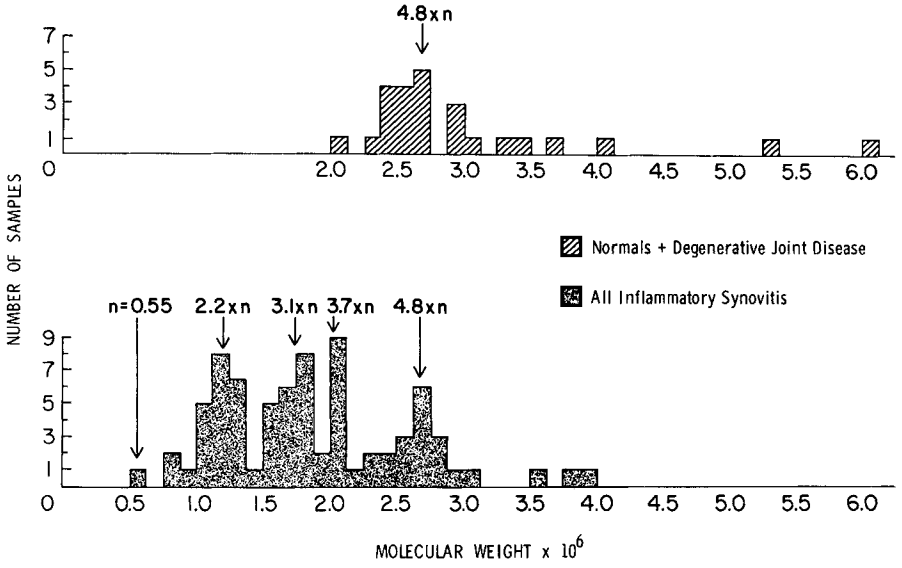

Fig. 1.—Frequency distribution of hyaluronate molecular weights. The arrows are placed at the arithmetic means of the different components of the frequency distribution.

Conversely, a white cell count of 500 cells/ml.[3] was associated in one fluid with an intrinsic viscosity of 19.6.

Synovial fluids from 30 patients with effusions of inflammatory origin, including rheumatoid arthritis, rheumatoid spondylitis, ulcerative colitis, and Reiter's syndrome, were examined for evidence of correlation between the concentration of white cells and intrinsic viscosity measurements. The mean white blood cell count in the synovial fluids was 14,230/mm.[3], while the mean intrinsic viscosity for the group was 26.4 dl./Gm. The correlation coefficient for these two sets of independent variables was calculated as: $r = -0.2022$, a correlation coefficient which does not suggest a significant association of intrinsic viscosity and joint fluid WBC count ($P > 0.1$).

The Frequency Distribution of Hyaluronic Acid Molecular Weights

A frequency distribution diagram of HA molecular weight is presented in Figure 1. While the fluid from normal persons and patients with degenerative joint disease approximates a normal distribution, such a distribution is not apparent for the "inflammatory" fluids. Instead the molecular weights of HA from inflammatory effusions are arranged in four distinct peaks. The mean molecular weights of the different peaks are approximately whole number multiples of the minimum molecular weight seen in this material (MW = 0.55×10^6).

Studies of Joint Fluid Characteristics During Re-formation of Synovial Effusions

Patient L. S., a 64-year-old white male patient with definite rheumatoid arthritis and effusion in both knees, was studied in the Clinical Research Unit of the Medical Center. In order to study the physical characteristics of re-forming effusion fluid, joint fluid aspiration was carried out repeatedly at 12 hour intervals. After baseline studies were completed, one joint was treated repeatedly with intra-articular hydrocortisone (37.5 mg. hydrocortisone tertiary butyl acetate). The characteristics of the steroid treated and control effusions were then compared at 12 hour intervals.

Figure 2 illustrates the changes in volume of joint fluid from the two knees noted during the study. The right knee exhibited a marked decrease

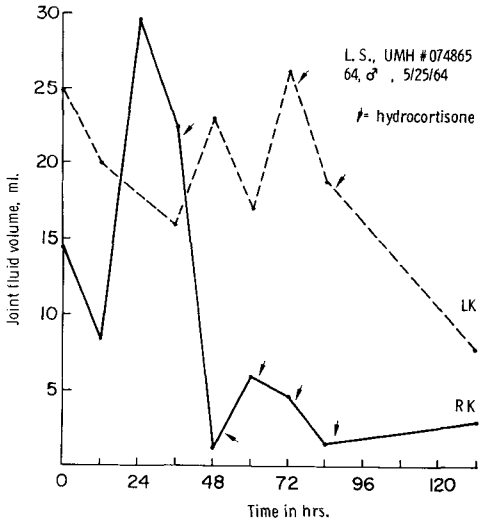

Fig. 2.—The placement of the arrows signifies that hydrocortisone was introduced into the joint cavity immediately following fluid aspirations represented by the connected dots.

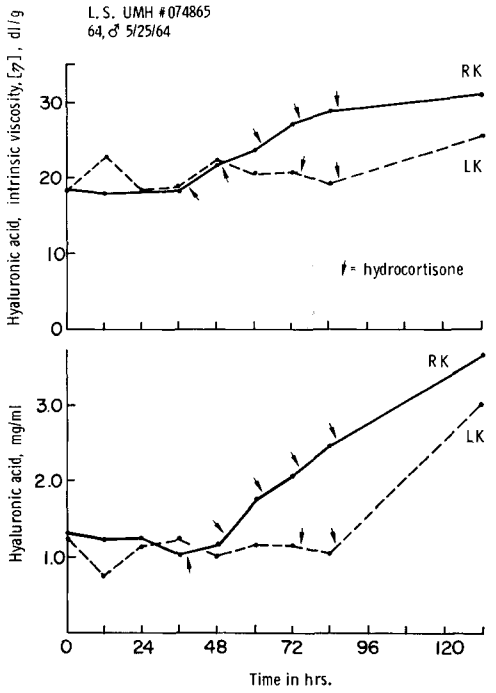

Fig. 3.—Hyaluronate concentration shows a threefold increase in response to local steroid, while polymer size approximately doubled. Note the gradual rate of change of intrinsic viscosity.

in joint fluid recoverable by needle aspiration coincident with the introduction of hydrocortisone. The reduction in joint fluid volume persisted, while the control, or left knee, persistently produced a large effusion in the range of the initial aspirate volume until the 72 hour point, when hydrocortisone was introduced with subsequent decrease of the fluid volume in that knee as well.

Changes in HA concentration and intrinsic viscosity are recorded in Figure 3. It is apparent that the HA in the right knee (repeatedly treated with hydrocortisone) showed a slow and rather modest increase in intrinsic viscosity. In the left knee (treated with hydrocortisone late in the study) the increase in intrinsic viscosity was of even lesser magnitude. More striking than an increase in intrinsic viscosity was the evidence for a rapid increase in HA concentration. As Figure 3 shows, the knee treated initially with steroids exhibited a rapid rise in HA concentrations to normal levels at 132 hours. The control knee showed maintenance of control levels of HA until the introduction of steroids late in the study, when it too showed an increase in concentration. The appearance rate (net) of HA in the right knee prior to introduction of hydrocortisone was 2.11 mg./hr., and this decreased to 0.52 mg./hr. after institution of hydrocortisone treatment. In the left knee the appearance rate of HA in the synovial cavity was 1.80 mg./hr., falling to 0.72 mg./hr. after intra-articular steroid.

Variation in the joint fluid white count during the 5½ days of repeated aspirations is illustrated in Figure 4. In the knee treated first with steroid, there was a downward trend in the white cell concentration, whereas in the control knee the white cell count rose rather precipitously at about 72 hours, coincident with increased clinical evidence of joint irritability, probably related to the trauma of repeated aspirations.

Patient F. D., a 64-year-old white male, was admitted to the Clinical Research Unit of the University of Michigan Medical Center for repeated aspiration of an olecranon bursal effusion. As illustrated in Figure 5, the rate of re-formation of bursal fluid was relatively constant at approximately 16.0 ml./12 hr. After the fifth aspiration, aqueous colchicine was injected into the bursa in an attempt to improve the diffusion barrier and reduce effusion.* Twelve to 14 hours

*In vitro studies indicate that human connective tissue cell cultures synthesize HA of high molecular weight when exposed to colchicine.[7] The role of macromolecular HA as a "diffusion barrier" in the synovial membrane is not proven.

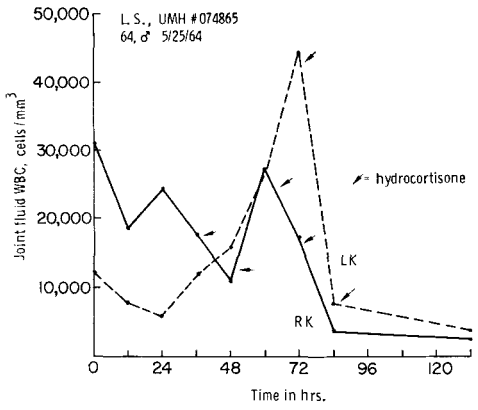

Fig. 4.—Changes in white blood cell concentration are similar to those seen in fluid volume.

after injection of colchicine the patient developed an acute exacerbation of localized synovitis lasting approximately 40 hours. Although there was a transient rise in the joint fluid white cell count, associated with warmth and discomfort over the bursa, the quantity of fluid was not appreciably changed by the increase in the inflammatory process.

Data concerning HA concentration and intrinsic viscosity during the recurrent bursal effusion is recorded in Figure 6. The HA concentration remained essentially unchanged during most of the study. Installation of hydrocortisone during the period of acute synovitis had no immediate affect on HA concentration or its net appearance rate, which remained approximately 1.0 mg./hr. It was of interest that the intrinsic viscosity of the HA was transiently elevated (probably beyond laboratory error) in the 12 hours immediately following the local placement of colchicine. However with the subsequent development of chemical synovitis, the intrinsic viscosity fell to lower than precolchicine levels, recovering seven days after administration of the colchicine. There was no evidence of an immediate affect of the hydrocortisone on the intrinsic viscosity of newly formed bursal fluid in the presence of acute synovitis.

Data concerning the total protein, albumin, and gamma globulin in the effusion fluid are presented in Figure 7. Total protein values were approximately 4.0 Gm. per cent throughout the investigation. There was a tendency for albumin to account for a progressively larger proportion of the joint fluid protein after repeated aspirations. Joint fluid sodium and potassium determinations done every 12 hours mirrored those of the plasma. It was of interest, on the other hand, that the joint fluid calcium was approximately 7.3 mg. per cent at the outset of the investigation with a gradual drop over the succeeding 5 or 6 days to a value of 6.6 mg. per cent on the final fluid specimen, compared with a plasma value of 8.9 mg. per cent.

Discussion

The concept that decreased joint fluid HA concentration normally occurs with advancing age is supported by the similarity of our values and those calculated from Hamerman's hexosamine data. The mean volume of fluid recovered from the knee joints of older normal persons was approximately 0.50 ml., compared with an average

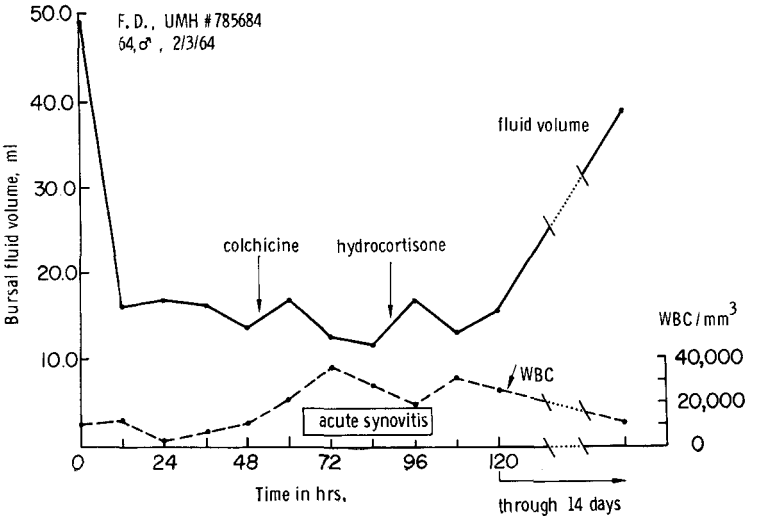

Fig. 5.—The anatomic characteristics of this bursa allowed reasonable certainty concerning complete fluid removal, and maximum distended volume corresponded with calculations based on geometric measurements.

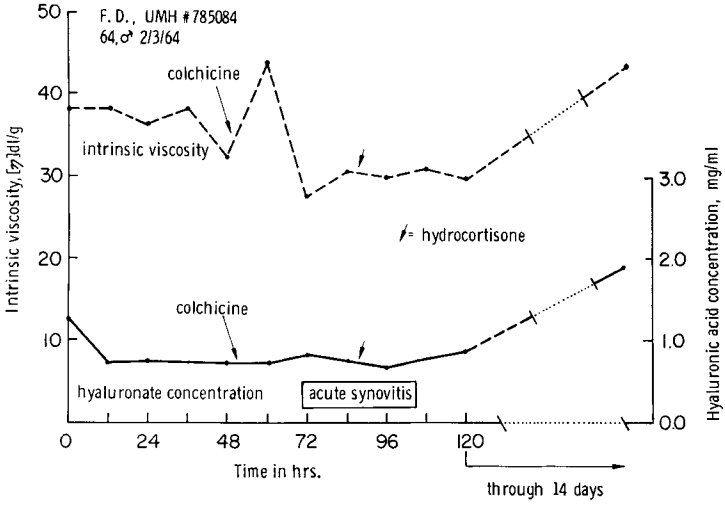

Fig. 6.—Colchicine induced a transient increase in HA molecular weight, and hydrocortisone had no measurable effect.

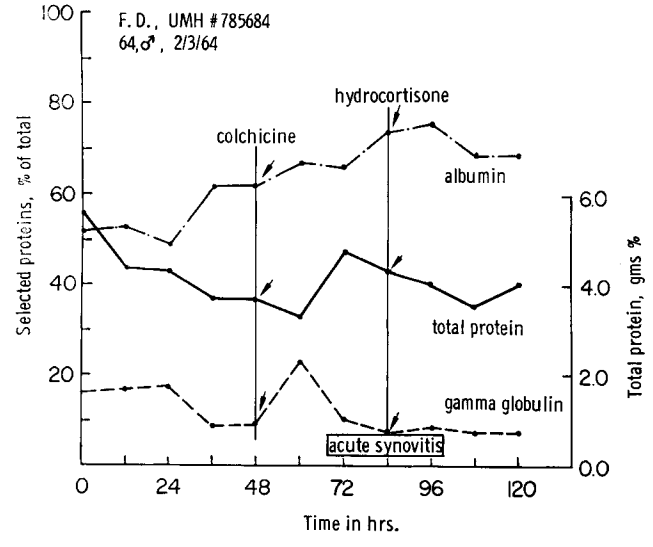

Fig. 7.—The total globulin component of joint fluid protein decreased in spite of the presumably permeable nature of the capillary bed in the inflamed synovium.

volume of 0.76 ml. in the younger normal group. We recognize that completeness of fluid removal from a complex joint like the knee is variable and hence we are not certain that decreased fluid volume is a consistent characteristic of the older joint, but by the same token it is clear that *there is no evidence showing an increased joint fluid volume* associated with aging. We may, then, conclude that not only HA concentration but total intracavitary HA is reduced in the older person. It is not clear whether decreased synthesis, accelerated degradation, or both, causes this decrease in joint fluid polysaccharide, a result which might accelerate degeneration of articular cartilage. Histologic study of normal synovial membranes from different age groups showed no age-related change in the frequency of subsynovial blood vessel cross sections, synovial intimal cells, mast cells, and subsynovial connective tissue cells when examined by a standardized counting procedure.[16] On the other hand, there was a 50 per cent reduction in number of endothelial cell nuclei seen ($P < 0.05$) on sections from persons over 30 years of age. If the approximately 50 per

cent reduction in joint fluid HA results from decreased synthesis, it appears to be on the basis of decreased HA formation by individual cells since synovial cell density appears unaffected. Whether decreased HA synthesis/cell in the aged person is related to decreased transport of nutritive materials dependent on endothelial cell activity or to reduction of a more specific interaction between endothelial and synovial intimal cells is a matter for speculation. It is pertinent that cell culture studies show equivalent HA synthesis capacity (rate/cell) in articular cells from young and older persons.[17-19] Other investigators have demonstrated that transport of simple sugars from capillary lumen to joint cavity is governed by components of the synovial membrane,[20] although neither the mechanism(s) nor responsible cell type(s) is known. We conjecture that reduced joint HA in older patients may result from hypofunction of individual cells, perhaps related to cellular alterations in the capillary bed.

Ogston's physicochemical studies lead him to doubt the simple random coil model for hyaluronic acid, suggesting instead a branched structure with partial cross-linking to form a "cage."[11] In the light of this possibility, the molecular weight distribution of HA from our patients assumes added interest. On the basis of present data, it might be argued that the common molecular species in human joint fluid are composed of 1 to 5 chains, each with a molecular weight of approximately 0.55×10^6. In view of the relatively small number of determinations, it is important to note that such a frequency distribution could occur by chance. The validity of the hypothesis may be further examined by (1) expanding the sample size; (2) fractionation of HA in joint fluid pools; (3) and possibly by enzymatic degradation of high molecular weight material to a basic subunit.

Eighty-two pathologic fluid specimens were characterized by increased volume and total intracavitary HA. In 10 instances HA concentration and molecular weight (MW) were both normal; in 14 cases only HA concentration was depressed; in 53 fluids both concentration and MW were decreased; and in 5 samples MW was low in the presence of borderline normal HA concentration. Such data are consistent with the possibility that discrete levels of articular cell dysfunction are correlated with either the intensity or stage of the "inflammatory process." The most minor abnormality of function might result from sufficient tissue trauma to induce increased capillary permeability and fluid transudation into the joint cavity. In this circumstance the joint might be expected to show only increased fluid volume, with normal HA concentration and molecular weight. As the severity of the process increased, the rate of fluid transfer to the joint would surpass the synthetic capacity of the synovial lining cells, and HA concentration would fall below normal. When the inflammatory process became sufficiently disruptive, joint lining cells would not only fail to maintain HA concentration, but would fail to maintain normal polymer weight as well. The five samples with low HA molecular weight as the dominant abnormality do not support the concept of an ordered biphasic response to the events of inflammation. It is pertinent, however, that the HA concentration was at the lower limits of normal in each instance and that these rather uncommon findings occurred in long-standing rheumatoid arthritis in which defective lymphatic removal of colloidal material might operate to increase HA concentration.

In the patient with bilateral knee effusions, local injection of hydrocortisone induced a prompt but disproportionate reduction in fluid volume and total intracavitary HA with a consequent rise in HA con-

centration. In both patients L. S. and F. D. there was little evidence of acute increase in HA polymer size induced by local steroid. The deferred increase in intrinsic viscosity was better correlated with the clinical evidence that synovitis was subsiding, suggesting that any positive affect which steroids may have had on polymer size was relatively indirect. Patient F. D. illustrates the potent disruptive potential of acute synovitis as it may be reflected in hyaluronate metabolism. During the 12 hours that clinical signs of acute synovitis were developing, the molecular weight of newly formed HA fell from a base line of about 2.75×10^6 to 2.02×10^6.

While most evidence indicates that low molecular weight hyaluronate is characteristic of inflammatory synovial effusions, it is uncertain whether this results from defective synthesis or intracavitary degradation. Serious consideration of intracavitary degradation of hyaluronate by white blood cell enzymes is discouraged by the absence of correlation between the number of WBC in the fluids and the intrinsic viscosity of the HA in the effusions.

Intra-articular hydrocortisone depressed the net HA synthesis rate 40 per cent and 75 per cent in the left and right knees, respectively, of patient L. S., values similar to those in synovial cell culture studies in which glucocorticoid excess depressed the HA synthesis rate by 50 to 75 per cent.[7,19,21,22] Patient F. D. falls into the group noted by Seppälä in which local steroid hormone treatment produced neither clinical change nor alteration in hyaluronate polymer size. It is not clear whether this defective response relates to rapid removal of the drug from the joint (thus precluding adequate exposure) or whether some specific "antisteroid" effect is operative in some circumstances.

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Summary

A decline in joint fluid hyaluronate concentration as a function of advancing age has been confirmed by an independent method. Synovitis due to a wide variety of causes was shown to be associated with decreased HA concentration and molecular weight. It is suggested that decreased HA concentration reflects primarily the vascular aspects of synovial inflammation, and decreased molecular weight is more directly related to altered function of the synovial lining cells. A multimodal distribution of hyaluronate molecular weights was noted and its possible significance discussed.

Summario in Interlingua

Le occurrentia de un declino in le concentration de hyaluronato in le liquido del articulationes in correlation con le avantiamento del etate ha essite confirmate per medio de un methodo independente. Synovitis occasionate per un extense varietate de causas se monstrava associate con un reduction del concentration e del peso molecular de acido hyaluronic. Es suggestionate que un reducite concentration de acido hyaluronic reflecte primarimente le aspectos vascular de inflammation synovial e que un declino del peso molecular es relationate plus directemente con un alterate functionamento del cellulas de revestimento synovial. Esseva notate un distribution multimodal de pesos molecular de hyaluronato, e le signification possibile de iste constatation es commentate.

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