HYALURONATE IN NORMAL HUMAN SYNOVIAL FLUID[1]

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Studies on hyaluronate in normal human synovial fluid have been hindered by the high viscosity and small volume of fluid available. Reports on hyaluronate levels in normal synovial fluid, in which a number of different methods have been used, have shown discordant results (1–4).

Several methods were used in the present study to determine the hyaluronate content of normal synovial fluid. Weighing rather than pipetting the small samples of viscous fluid made it possible to measure hyaluronate in 0.25 Gm. of synovial fluid. Hyaluronate was precipitated in mucin clots formed by adding acid or a polyvalent cation to synovial fluid, and was measured indirectly by determining hexosamine in these clots. Since a part of the hexosamine of synovial fluid is not a component of hyaluronate, an important aspect of this work was the demonstration that no non-hyaluronate hexosamine was precipitated in mucin clots, and that no hyaluronate hexosamine remained in the supernatant after a mucin clot was formed. Hyaluronate content of synovial fluid was also measured by determining the fall in hexosamine after hyaluronidase digestion and dialysis of whole synovial fluid. Figures for hyaluronate in synovial fluid as determined by mucin clot technique, and by hyaluronidase digestion and dialysis of synovial fluid agreed, lending confidence in the validity of these methods.

MATERIALS AND METHODS

Deceased subjects were selected who died suddenly after traumatic injury or a vascular accident and who showed no obvious joint disease or systemic illness that might contribute to pathological joint effusions. Synovial fluid was obtained with sterile precautions from both knee joints within six hours after death. Fluids were frozen at −20° C. unless studied within 24 hours, in which case they were stored at 5° C.

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I. Hexosamine determinations on synovial fluid and mucin clots

Synovial fluid. Samples of the viscous synovial fluid were weighed rather than pipetted. Aliquots of synovial fluid (0.10 to 0.15 Gm.) were transferred in glass droppers with tapered ends to pyrex test tubes (13 by 100 mm.). The synovial fluid was diluted to 1 ml. with distilled water and 1 ml. of 8 N HCl was added. The tubes were sealed with a gas-oxygen flame.

Mucin clots. Hyaluronate and protein were precipitated from synovial fluid as mucin clots. Three methods of making mucin clots were studied.

a) Glacial acetic acid. The method of Ropes, Robertson, Rossmeisl, Peabody, and Bauer (5) for bovine synovial fluid was modified. Synovial fluid (0.2 to 0.4 Gm.) was weighed in pyrex test tubes (13 by 100 mm.) and diluted 1:5 with tap water. The diluted synovial fluid was mixed and the tubes placed in a cold room (5° C.) for 15 minutes. A mucin clot was formed in the cold upon addition to the tubes of a volume of glacial acetic acid equal to 1 per cent of the total volume of diluted synovial fluid. The clot adhered to a glass stirring rod used to mix the fluid. After 20 minutes the clots were detached from the stirring rod and the clot and clear supernatant in the test tube centrifuged at room temperature. The supernatant fluid was decanted and the precipitated mucin was washed once with cold distilled water, once with absolute alcohol and dried over P_2O_5 in a vacuum. Two ml. of 4 N HCl was added and the tubes were sealed.

b) Ten per cent (v/v) acetic acid (6). The above procedure was followed. A volume of 10 per cent acetic acid equal to 2.5 per cent of the total volume of 1:5 diluted synovial fluid was used to form a mucin clot.

c) Hexaethylenediamine-hexoltetracobalt (III) nitrate, hereafter referred to as brown salt, is a brown cobalt salt recrystallized three times (7). The cation has a charge of +6 and its formula is

$$Co\left(\begin{matrix}HO\\HO\end{matrix}>Co\ en_2\right)_3\ (NO_3)_6.$$

Synovial fluid (0.2 to 0.4 Gm.) was weighed in pyrex tubes (15 by 125 mm.) and diluted 1:20 with tap water. A volume of a 1 per cent (w/v) solution of brown salt was added equal to 6 per cent of the total volume of diluted synovial fluid.

The sealed tubes were hydrolyzed at 100° C. for six and one-half hours. The tubes were opened, the contents were filtered through Whatman No. 1 paper and were diluted to 25 ml. with distilled water. Aliquots were taken for hexosamine and nitrogen determinations.

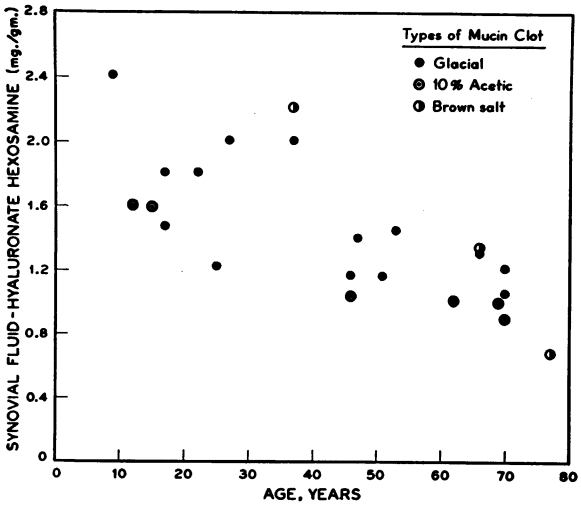

FIG. 1. LEVELS OF HYALURONATE HEXOSAMINE IN SYNOVIAL FLUID OF NORMAL SUBJECTS AT VARIOUS AGES

Hexosamine was determined by the Elson and Morgan procedure as modified by Bilow and Hatch (8). The Kjeldahl procedure (9) was used for nitrogen analysis.

The error in duplicate mucin precipitations and hexosamine analyses was less than 5 per cent.

II. Hyaluronidase digestion of synovial fluid and mucins

Preparation—Synovial fluid. The hyaluronate hexosamine content of synovial fluid was determined by forming a mucin clot and measuring the hexosamine in the clot. Aliquots (0.5 to 1.0 Gm.) of synovial fluid were then diluted by addition of buffer (0.1 M NaCl and 0.1 M $NaHCO_3$, pH 8.1) to reduce the hyaluronate hexosamine content of the synovial fluid to about 0.28 mg. per Gm. The diluted synovial fluid was dialyzed in buffer at 5° C. for three days with daily changes of buffer. Hexosamine was determined in 0.25 Gm. aliquots of this diluted dialyzed synovial fluid and the hyaluronate hexosamine of this fluid was calculated as follows (hexosamine is abbreviated as hx.):

Hx. of diluted dialyzed synovial fluid × per cent hyaluronate hx. in whole synovial fluid equals hyaluronate hx. of diluted dialyzed synovial fluid.

Preparation—Mucins. Aliquots of synovial fluid were weighed in flat bottom centrifuge tubes (40 ml. capacity) and mucin clots were formed by the techniques described. The mucin clots were washed twice with cold distilled water. The hexosamine concentration was adjusted to 0.28 mg. per Gm. by adding buffer, and the mucins were dissolved in the buffer by constant stirring for 24 hours at 5° C. The hexosamine content of dissolved mucin was equivalent to hyaluronate hexosamine. After determination of their viscosity, and prior to hyaluronidase digestion, the mucin solutions were dialyzed against buffer for two days at 5° C.

Preparation—Supernatants remaining after mucin precipitation. The mucin supernatants were first dialyzed against buffer for two days at 5° C. NaCl was added to the supernatant of the brown salt mucin so that excess cobalt would be displaced during dialysis. Following dialysis in buffer, all supernatants were dialyzed at 5° C. for two days in distilled water. The contents of the dialysis casing were emptied into beakers and evaporated to dryness over P_2O_5 *in vacuo*. Buffer was added to the beakers and the supernatant material dissolved by mixing. The hexosamine concentration of this material was about 0.2 mg. per Gm. or less.

Hyaluronidase digestion. Three ml. of diluted dialyzed synovial fluid or dissolved dialyzed mucins was brought to pH 4.7 to 4.8 with 10 per cent acetic acid. Glacial acetic acid was used to lower the pH of the mucin supernatants since it was necessary to avoid further dilution of the small quantity of hexosamine in the supernatants. The acidified fluids were then divided into three parts by distributing 1 ml. aliquots to a stoppered glass vial (8 ml. capacity). The contents of one vial served as a control and 0.1 ml. of the NaCl–$NaHCO_3$ buffer was added. To the contents of a second vial was added 0.1 ml. of the same buffer containing 75 turbidity reducing units (TRU) of testicular hyaluronidase (Wyeth). One-tenth ml. of this buffer containing 75 TRU of streptococcal hyaluronidase was added to the fluid in a third vial. The vials were shaken and then incubated at 37° C. for 72 hours. The contents of the vials were then thoroughly mixed and dialyzed on a shaker platform in three separate beakers at 5° C. for three days with changes of buffer twice daily. Aliquots up to 1.0 Gm. of the dialyzed material were weighed in pyrex tubes (13 by 100 mm.) and hexosamine determinations were carried out. Hexosamine of the dialyzed control less hexosamine of the hyaluronidase treated dialyzed material was a measure of hyaluronate hexosamine.

Hyaluronidase activity was also tested against well defined substrates to define accurately such variables as the extent of enzymatic digestion and the hexosamine blank contributed by the enzymes. These substrates included: 1) 4 per cent (w/v) gamma globulin dialyzed against buffer; 2) 1 ml. (50 mg.) of heparin (Vitarine Co.) diluted to 50 ml. with buffer; 3) hyaluronate prepared (5) from bovine synovial fluid, partially purified (hexosamine, 23 per cent; nitrogen, 6 per cent) and dissolved in buffer to a concentration of about 0.3 mg. hexosamine per Gm.; and 4) potassium chondroitin sulfate (24 per cent hexosamine) prepared from the crystalline calcium salt and similarly dissolved.

III. Viscosity measurements

These studies were carried out on diluted dialyzed synovial fluids and on dissolved mucins at comparable hyaluronate hexosamine concentrations (0.28 mg. per Gm.). Viscosity of these materials was measured at 37° C. using Ostwald viscosimeters of 2 ml. capacity and a flow time for buffer of 24 seconds.

IV. Bovine synovial fluid

Studies of this material form a minor part of this report. Fluid obtained from the astragalo-tibial joints of freshly slaughtered animals was clarified by filtration through glass wool and stored at − 20° C. The concentration of hyaluronate hexosamine in this fluid was about 0.18 mg. per Gm. Before studies of the extent of hyaluronidase digestion were carried out, bovine fluid was concentrated *in vacuo* until the hyaluronate hexosamine concentration was raised to about 0.28 mg. per Gm., and was then dialyzed in buffer.

RESULTS

I. Analytical data

Table I presents the total hexosamine of synovial fluids, and hexosamine in mucin clots precipitated from synovial fluids of the 23 normal subjects. Total nitrogen, mucin nitrogen and nitrogen to hexosamine ratios were measured in some subjects. In all cases but two, mucin hexosamine, calculated as mg. per Gm. of synovial fluid, was lower than the total hexosamine of synovial fluid. As shown in data presented below, mucin clots formed from synovial fluid contained all the hyaluronate hexosamine and only non-hyaluronate hexosamine remained in the supernatant. A figure for hyaluronate in synovial fluid may be obtained by dividing mucin hexosamine by 40 per cent, the hexosamine content of protein free hyaluronate. Where the three methods used to form mucin clots can be compared, figures for hexosamine in the clots are similar. Ten per cent acetic acid at times led to incomplete precipitation of the hyaluronate as judged by lower mucin hexosamine values. There were slight differences in the analysis of the clots with respect to the nitrogen to hexosamine ratios.

Levels of hyaluronate hexosamine in normal synovial fluid of subjects ranging in age from 9 to 77 years are shown in Figure 1. Values prior to age 40 were somewhat higher than the hyaluronate hexosamine levels after this age.[2]

II. Hyaluronidase digestion

A necessary condition for the digestion of susceptible substrates to *dialyzable* units by testicular hyaluronidase was that the pH of these substrates be below 5 (70 year old subject, Table II). At higher pH levels, testicular hyaluronidase markedly reduced the *viscosity* of the substrates but did not digest the substrates to dialyzable units. Material to be digested by hyaluronidases was adjusted to a pH of about 4.7. Streptococcal hyaluronidase depolymerized susceptible substrates to dialyzable units over a broader pH range, but was somewhat more effective in this respect at pH 4.7 than at 6.8.

TABLE I

Hexosamine and nitrogen in normal synovial fluid at various ages *

Age	Total hx.	Hyaluronate hx. clot	Hyaluronate hx. mg./Gm.	Total N.	Mucin N.	N./hx. ratio
	mg./Gm.	*clot*	*mg./Gm.*	*mg./Gm.*	*mg./Gm.*	
9	2.59	Gl.	2.42			
12	1.61	10% A.	1.61	3.34	2.05	16.4
15	1.60	10% A.	1.60		2.18	17.5
		Br.	1.55			
17	1.71	Gl.	1.48			
		Br.	1.45			
17	2.05	Gl.	1.82			
		Br.	1.75			
22		Gl.	1.82		2.28	16.1
	2.26	10% A.	1.81	4.35		
		Br.	1.84			
25		Gl.	1.23		1.49	15.5
	1.46	10% A.	1.27	3.23	1.85	18.7
		Br.	1.20		1.44	13.4
27		Gl.	2.02		2.29	14.5
	2.03	10% A.	1.89	4.41	2.66	18.0
		Br.	1.86		2.73	16.8
37	2.53	10% A.	2.11			
		Br.	2.23			
37	2.28	Gl.	2.02			
46	1.39	10% A.	1.04	3.93	1.92	23.7
46	1.39	Gl.	1.17			
		Br.	1.18			
47		Gl.	1.41		1.71	15.5
	1.68	10% A.	1.48	3.14	2.21	19.2
		Br.	1.35		1.50	12.2
51		Gl.	1.17			
	1.47	10% A.	1.01			
		Br.	1.22			
53		Gl.	1.47			
	1.88	10% A.	1.49			
		Br.	1.48			
62	1.39	10% A.	1.02		1.72	21.5
66	1.58	10% A.	1.15			
		Br.	1.35			
66	1.80	Gl.	1.32	4.46	1.93	18.6
69	1.20	10% A.	0.99			
		Br.	1.00			
70	1.12	10% A.	0.89			
70		Gl.	1.22		1.54	16.1
	1.60	10% A.	1.26	3.46	2.02	20.4
		Br.	1.23			
70	1.37	Gl.	1.05			
77	1.01	10% A.	0.69	2.94	1.24	23
		Br.	0.68		1.29	22

* Abbreviations are as follows: Hx., hexosamine; N., nitrogen; Gl., glacial acetic acid; 10% A., 10% acetic acid; Br., brown salt.

[2] Since submitting this paper for publication, additional determinations of hyaluronate hexosamine levels in synovial fluid have been made: Age 33, 1.98 mg. per Gm.; age 52, 1.53 mg. per Gm.; age 85, 0.79 mg. per Gm.

TABLE II

Results of hyaluronidase digestion of synovial fluids and mucins *

Age	Sample	[1]†	pH	Control hx.	Enzymatic digestion and dialysis: After 75 TRU T. Hyd. hx.	Hx. rendered dialyzable	After 75 TRU S. Hyd. hx.	Hx. rendered dialyzable
				mg./Gm.	*mg./Gm.*	%	*mg./Gm.*	%
	Chondroitin sulfate		4.8	0.220	0.008	97	0.227	0
	Bovine hyaluronate		4.8	0.240	0.018	93	0.032	87
	Heparin		5.2	0.141	0.151	0	0.155	0
	γ Globulin		4.8	0.419	0.410	0	0.412	0
17	Synovial fluid	86	6.8	0.296			0.261	12
	Br. mucin		6.8	0.272			0.045	83
22	Synovial fluid	81	4.8	0.279	0.046	84	0.065	77
	Gl. mucin		4.7	0.230	0.017	93	0.030	87
	10% A. mucin		4.8	0.243	0.024	90	0.035	86
	Br. mucin		4.7	0.253			0.033	87
25	Synovial fluid	85	4.6	0.250	0.040	84	0.079	69
47	Synovial fluid	84	4.8	0.343	0.044	87	0.161	54
66	Synovial fluid	73	4.8	0.334	0.111	67	0.278	17
70	Synovial fluid	76	6.5	0.272	0.217	20	0.077	72
	Synovial fluid	76	4.7	0.255	0.067	74	0.077	70
	Gl. mucin		6.8	0.258			0.049	81
	Gl. mucin		4.7	0.240	0.027	89	0.030	88
	10% A. mucin		4.7	0.228	0.024	90	0.028	88
	Br. mucin		4.7	0.253	0.021	92	0.031	88
	Gl. sup't		4.7	0.124	0.120	3		
	10% A. sup't		4.5	0.177	0.165	7		
	Br. sup't		4.6	0.122	0.117	4		

* Abbreviations are as follows: T. Hyd., testicular hyaluronidase; S. Hyd., streptococcal hyaluronidase; sup't, supernatant; TRU, turbidity reducing units.

† [1] equals per cent of total hexosamine of synovial fluid that is hyaluronate hexosamine determined by hexosamine in glacial acetic acid mucin.

Standard substances. A number of experiments were carried out to test the specificity of the hyaluronidases and the extent to which they hydrolyzed known substrates. Table II shows these results. Protein free chondroitin sulfate was digested to the extent of 97 per cent with testicular hyaluronidase but was not affected by the streptococcal enzyme. Hyaluronate prepared from bovine synovial fluid, partially purified (nitrogen to hexosamine ratio of 3) but still containing some protein, showed a fall in hexosamine of 93 per cent after testicular hyaluronidase digestion and 87 per cent after the use of the streptococcal enzyme. This apparent difference in the degree of digestion of hyaluronate by the two enzymes may represent a somewhat greater artificial "blank" in hexosamine analysis contributed by the streptococcal enzyme itself, and probably does not represent less complete digestion of the substrate by streptococcal hyaluronidase. The mucopolysaccharide heparin was not digested by either of the hyaluronidases. Absence of proteolytic activity of the hyaluronidase preparations was shown by their lack of effect in reducing hexosamine content of gamma globulin.

Synovial fluid. Incubation of synovial fluid with hyaluronidase and subsequent dialysis removed that part of the total hexosamine that was hyaluronate hexosamine. The extent of digestion agreed with the estimate of hyaluronate hexosamine in synovial fluid as determined by hexosamine in mucin clots. Table II gives this data and shows that there is very close agreement between the hyaluronate hexosamine of synovial fluid as estimated by glacial acetic acid mucin clots, and that estimated by the extent of digestion of synovial fluid with testicular hyaluronidase at pH 4.7. Streptococcal hyaluronidase in some cases gave very similar results, but at times, as shown in the cases of the 17 and 66 year old subjects, incompletely digested hyaluronate in synovial fluid. The reason for this is not certain but may be due to the presence in these fluids of antibodies to streptococcal hyaluronidase. The very remote

possibility that hyaluronate was not the anionic mucopolysaccharide in these fluids was excluded by noting that streptococcal hyaluronidase completely digested mucins precipitated from these synovial fluids (17 year old subject, Table II).

Mucins. Mucin clots contained all the hyaluronate hexosamine of synovial fluid. Hexosamine in mucins was digested to dialyzable units by streptococcal hyaluronidase. The extent of digestion of the mucins by streptococcal and by testicular hyaluronidase corresponded exactly to that observed in the digestion of purified bovine hyaluronate (Table II).

Further evidence that all the hyaluronate was precipitated from synovial fluid when mucin clots were formed was shown by failure of hyaluronidase digestion and dialysis to reduce the hexosamine in the remaining supernatants (70 year old subject, Table II). The possibility was tested that hyaluronidase activity was inhibited in the supernatant fluid. Glacial acetic acid mucin added to a supernatant was digested by hyaluronidase to the extent of 90 per cent.

III. Viscosity studies

Relative viscosities of synovial fluids diluted with buffer and dialyzed against buffer and mucins dissolved in buffer are shown in Table III. These studies were carried out on fluids of the same ionic strength, pH, and hyaluronate hexosamine concentration. The data show no apparent change in viscosity with aging, but the limitations of this type of measurement in detecting minimal but critical changes in hyaluronate are recognized (10).

Brown salt mucin had a higher relative viscosity than mucins prepared by acidification of synovial fluid. At equivalent hyaluronate hexosamine concentrations the viscosity of the brown salt mucin was equal to the viscosity of the original synovial fluid. Binding of cobalt ions by the brown mucin did not contribute to the higher viscosity noted; after addition of NaCl and dialysis against buffer to remove cobalt ions, the viscosity of the brown mucin was unchanged.

TABLE III

Relative viscosities of normal synovial fluids dialyzed in buffer, and mucins dissolved in buffer, at equivalent hyaluronate hexosamine concentration

Age	Dialyzed synovial fluid: Total hx.	Dialyzed synovial fluid: Hyaluronate hx.*	η	Dissolved mucins	Hx.	η
	mg./Gm.	*mg./Gm.*			*mg./Gm.*	
17	0.31	0.29	7.3	Br.	0.26	5.4
17	0.32	0.28	7.3			
				Gl.	0.27	3.4
				10% A.	0.29	3.7
22	0.36	0.29	7.4	Br.	0.30	7.0
				Gl.	0.28	3.8
27	0.28	0.28	5.8	10% A.	0.28	4.8
				Br.	0.28	5.7
37				Gl.	0.29	5.1
46	0.33	0.28	6.4			
66	0.41	0.28	5.5			
				Gl.	0.29	5.4
70	0.34	0.26	6.8	10% A.	0.27	5.0
				Br.	0.29	6.8

* Calculated from the per cent of the total hexosamine of synovial fluid found in glacial acetic acid mucin clots.

IV. Bovine synovial fluid

The results of a number of experiments on bovine synovial fluid are summarized in Table IV. On the basis of the fall in hexosamine following hyaluronidase digestion and dialysis of synovial fluid, hyaluronate hexosamine accounted for a little less than one-half the total hexosamine of synovial fluid. Non-anionic mucopolysaccharides containing hexosamine were precipitated to some extent in mucins, particularly those formed with 10 per cent acetic acid and brown salt. The nitrogen to hexosamine ratio of brown salt mucin

TABLE IV

Studies on bovine synovial fluid

	Hx.	% of total synovial fluid hx. in mucin	Hx. rendered dialyzable after hyaluronidase: S. Hyd.	Hx. rendered dialyzable after hyaluronidase: T. Hyd.	Nitrogen	N./hx. ratio
	mg./Gm.		%	%	*mg./Gm.*	
Synovial fluid	0.37		47	45	2.18	
Gl. mucin	0.18	49	77	79	0.23	16.3
10% A. mucin	0.20	54			0.37	23.7
Br. mucin	0.20	54			0.65	39.5

TABLE V

Levels of hyaluronate in normal human synovial fluid

Reference	Hyaluronate	Hyaluronate hx.	Method
	mg./ml.	*mg./ml.*	
(1)	0.13		Turbidity
(2)	(2.17)*	0.87	Hexosamine determination in glacial acetic acid mucin clots
(3)	2.97	(1.19)	Hyaluronidase digestion of synovial fluid. Precipitation of non-hyaluronate hexosamine with trichloracetic acid and hexosamine determination on supernatant
(4)	1.71	(0.68)	Determination of hexuronic acid in synovial fluid after removal of some protein with chloroform
Present authors	(3.60)	1.44	Measurement of hexosamine in mucin clots
			Measurement of hexosamine in control and hyaluronidase digested and dialyzed synovial fluids

* Figures in parentheses are calculated from authors' data. The figure of 40 per cent hexosamine in hyaluronate is assumed.

shown in Table IV was higher than values of about 28 observed in earlier experiments on mucins similarly formed in fresh fluid. The higher ratio shown may represent increased precipitation of protein containing non-hyaluronate hexosamine when brown salt was used to form a mucin in fluids stored for some time.

DISCUSSION

An attempt has been made to provide accurate methods for determining hyaluronate levels in normal human synovial fluid. The first point which required attention was the method by which samples of synovial fluid were taken for analysis. Small volumes of highly viscous fluid could not be delivered accurately by pipetting. Measurements were therefore made on the basis of the weight rather than the volume of the synovial fluid. The density of diluted synovial fluid was found not to differ from water within the limits of the precision of the method. Grams and milliliters may therefore be used interchangeably.

All the hyaluronate hexosamine of synovial fluid was precipitated in a mucin clot formed by adding acetic acid or a polyvalent cation to synovial fluid. Non-hyaluronate hexosamine remained in the supernatant and accounted for about 15 per cent or less of the total hexosamine of joint fluid, as previously noted by Sundblad (3). This non-hyaluronate hexosamine was presumably a component of non-anionic mucopolysaccharides. For quantitative precipitation of hyaluronate, glacial acetic acid was preferred rather than 10 per cent acetic acid. A complex cobalt salt, termed brown salt in this paper, with a charge of + 6, also precipitated hyaluronate from synovial fluid. This salt was used by Shatton and Schubert in their work with bovine synovial fluid (11). Mucins precipitated by the brown salt and by glacial acetic acid were similar with respect to hexosamine content and nitrogen to hexosamine ratio. All the hexosamine in mucin clots was digested and rendered dialyzable by streptococcal hyaluronidase. No hexosamine was lost after hyaluronidase digestion and dialysis of mucin supernatants.

Determining the fall in hexosamine following hyaluronidase digestion and dialysis of whole synovial fluid was an additional method to measure accurately hyaluronate levels in synovial fluid. These results agreed closely with those obtained by the mucin clot method.

The average hyaluronate content of normal human synovial fluid in the cases studied was 3.6 mg. per Gm. This figure may be compared with data of other workers in Table V. The average hyaluronate level presented in this paper is higher than any of the figures of other workers. One reason for this may be due to weighing rather than pipetting samples of synovial fluid. Sundblad (3) estimated that the normal range of hya-

luronate in human synovial fluid was 2 to 4 mg. per ml. By contrast with normal human fluid, hyaluronate in bovine synovial fluid was 0.45 mg. per Gm. and hyaluronate hexosamine constituted about 45 per cent of the total hexosamine, as has been previously noted (12–14), instead of 85 per cent as found in human joint fluid. The physical properties of these fluids cannot be compared unless the hyaluronate levels are made equivalent.

Weighing samples of synovial fluid has made it possible to examine the fluid from individual knee joints of subjects of various ages. Generally higher values of hyaluronate have been noted in synovial fluid of subjects before the age of 40. These findings are of interest because articular cartilage of synovial joints has been shown chemically and histologically to contain less chondroitin sulfate with aging (15) or with degenerative arthritis (16–17). Workers whose methods are outlined in Table V have measured hyaluronate concentration in synovial fluid of patients with degenerative arthritis. Compared with normal figures, lower levels of hyaluronate [1.4 mg. per ml. (3)], equivalent levels [2.0 mg. per ml. (2)] and higher values [2.1 mg. per ml. (4)] have been found. Two patients with severe degenerative arthritis of the knees were studied by the present authors. Hexosamine was determined in mucin clots formed by adding glacial acetic acid to the synovial fluid of these two subjects. The accuracy of this technique for measuring hyaluronate in these pathological synovial fluids was established by methods similar to those used for normal fluids described in this paper. Hyaluronate levels of 2.0 mg. per ml. and 1.8 mg. per ml. were found in the joint fluid of these subjects aged 54 and 59, respectively. Values for normal subjects in this age range were about 3 mg. per ml. Articular cartilage and synovial fluid may thus show an analogous decrease in their anionic mucopolysaccharide concentration in the course of degenerative joint changes.

SUMMARY

1. Methods have been described to measure hyaluronate in very small quantities of human synovial fluid. Hyaluronate was precipitated in mucin clots formed by adding acid or a polyvalent cationic cobalt salt to weighed aliquots of synovial fluid and was measured indirectly by determining hexosamine in these clots.

2. These clots contained all the hyaluronate hexosamine of synovial fluid and all the hexosamine in these clots was digested and rendered dialyzable by streptococcal hyaluronidase. Non-hyaluronate hexosamine remained in the supernatant and was not digested by hyaluronidase.

3. Hyaluronate in whole synovial fluid was also determined by measuring the fall in hexosamine after hyaluronidase digestion and dialysis. These figures for hyaluronate agreed with those obtained by the mucin clot technique.

4. The average hyaluronate concentration of normal human synovial fluid was 3.6 mg. per Gm. Hyaluronate hexosamine constituted 85 per cent or more of the total hexosamine of synovial fluid. By contrast, hyaluronate content of bovine synovial fluid was 0.45 mg. per Gm. and hyaluronate hexosamine constituted about 45 per cent of the total synovial fluid hexosamine.

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