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Viscous interactions of hyaluronic acid with some proteins and neutral saccharides

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Synovial fluid greatly delays the centrifugal compression of collagen gels, which can be interpreted as a viscous effect of hyaluronic acid (Fessler, 1960). This effect is enhanced by adding serum, suggesting that proteins in turn significantly influence the viscosity of hyaluronic acid (Baxter, Fraser, and Harris, 1971).

Although the high viscosity of synovial fluid is primarily due to hyaluronic acid, the effect of proteins on the viscosity of hyaluronic acid is nevertheless not entirely clear. The non-Newtonian (*i.e.* shear-dependent) element of synovial viscosity seems to stem largely from a complex of hyaluronic acid with a small specific group of proteins (Ogston and Stanier, 1950, 1952; Silpananta, Dunstone, and Ogston, 1968, 1969), but any contribution to viscosity from the interaction of hyaluronic acid with the much greater residual fraction of synovial proteins is at present undefined.

A commonly held view is that the effect of protein is small and merely additive. Thus the viscosity of synovial fluid falls after treatment with hyaluronidase to a low level close to that of an equal concentration of serum proteins ($\eta_r^* \approx 1{\cdot}2$; Sundblad, 1953), and it has been found not to be significantly lowered by step-wise separation of proteins, when assessed by measurement or derivation of *intrinsic* viscosity; that is, in a state of limiting, or infinite, dilution (Balazs and Sundblad, 1959; Balazs, Watson, Duff, and Roseman, 1967). Brief observations in the course of other studies have shown no change in viscosity on addition of proteins to hyaluronic acid (Ropes, Robertson, Rossmeisl, Peabody, and Bauer, 1947; Ogston and Stanier, 1952; Johnston, 1955) and clinical references to the matter seem tacitly to agree with Balazs and Sundblad (1959), who concluded that in respect of viscosity the presence of proteins does not influence the behaviour of hyaluronic acid.

* η_r = relative viscosity, or viscosity ratio.

However, inferences based on measurement of intrinsic viscosity are not necessarily true of finite concentrations. Any material which alters the character of the solvent can change the viscosity of a solution in either direction (Alfrey, Bartovics, and Mark, 1942) and the viscosity of a mixture of homologous polymers can be assumed to be additive only at limiting dilution (Nichol, Bethune, Kegeles, and Hess, 1964). Ogston (1962) calculated, on the basis of his previous osmotic studies and other physical data, that very dilute hyaluronic acid should be sensitive to the presence of albumin although later observations of viscosity did not confirm this prediction (Preston, Davies, and Ogston, 1965).

The concentrations of proteins and hyaluronic acid in synovial fluid vary from joint to joint and between species, and most of all in disease. In view of the apparent conflicts in the references cited, the effects of mixing proteins and other viscous materials with hyaluronic have been studied in concentrations closer to those that occur naturally, primarily in terms of viscosity ratio since it is the derivative closest to absolute viscosity.

Materials

SYNOVIAL FLUID

Pooled samples, taken from carpal joints of cattle immediately after slaughter, were chilled and centrifuged at 25,000 G. for 30 to 60 min. Toluene was added as antiseptic, and aliquots were stored at −85°C. In the nine samples used, the hyaluronic acid content was $1{\cdot}5 \pm 0{\cdot}12$ mg./ml., and the protein content $15{\cdot}9 \pm 2{\cdot}4$ mg./ml.

SERUM

This was prepared from blood of fasting normal human subjects and stored as above.

BOVINE SERUM ALBUMIN, HUMAN GAMMA GLOBULIN

These were obtained from the Commonwealth Serum

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Laboratories, Melbourne. Each showed a single component on immunoelectrophoresis with antiserum against whole serum. The globulin consisted of immunoglobulin G, 90 per cent. as monomer (Dr. S. Sutherland, personal communication).

FICOLL

This was the sucrose polymer of Pharmacia Ltd., with a stated $\bar{M}_w$ of 400,000.

CHEMICALS

Glucose, sucrose, and polyethylene glycol 6000 were from British Drug Houses Ltd.; glycerol from E. Merck.

BUFFERS

0·05 M Sorensen's phosphate, pH 7·25 in 0·1 M NaCl was used in preliminary work; the other was a phosphate-buffered saline with potassium, calcium, and magnesium, according to Dulbecco and Vogt (1954), pH 7·25.

Methods

PURIFICATION OF HYALURONIC ACID

This was done by density gradient centrifugation of synovial fluid in CsCl at an initial density of 1·55. After 90 hrs at 105,000 G, in a Beckman Spinco 50 Ti rotor, the bottom 3 ml. of the 10–11 ml. in each tube were pooled and then exhaustively dialysed against 0·15 M NaCl, water, and finally buffer. Protein content relative to that of hyaluronic acid (w/v) in this fraction was less than 2 per cent. No attempt was made to separate the small amount of chondromucoprotein which would be expected in the foregoing modification of the original method of Silpananta and others (1968).

Hyaluronic acid was measured against glucuronolactone and hyaluronic acid standards by the method of Bitter and Muir (1962) with limited heating (Harris and Fraser, 1969). Protein was measured against crystalline bovine serum albumin standards by the method of Lowry, Rosebrough, Farr, and Randall (1951).

SOLUTIONS

Solutions of hyaluronic acid were thoroughly mixed by gentle rotation to avoid bubbling and denaturation of added proteins. Densities were determined by pycnometry with triple-distilled water as a standard. To permit use of the relatively weakly buffered Dulbecco-Vogt saline, the protein solutions, particularly albumin, required exhaustive dialysis to achieve a pH range of 7·1–7·4 where viscosity of hyaluronic acid is not affected by small differences in pH.

VISCOMETRY

Particulate matter was removed from solutions by preliminary ultracentrifugation. All measurements were made at 25°C.

Four BS/U capillary viscometers were used. A type M2 with a flow rate of 150 sec. and a calculated mean shear rate of 800 sec.$^{-1}$ for water at 25°C. was used for the initial studies only. The remainder were done with type M4 viscometers with corresponding flow times of 24 sec. and shear rates of 1300 sec.$^{-1}$. A Hewlett Packard Auto-viscometer was used for timing wherever possible. All instruments were calibrated by plotting $\eta/\rho t$* against $1/t^2$ with water at four temperatures. Kinetic energy corrections were zero, and the three M4 instruments were closely matched as shown by the intercepts of $\eta/\rho t$ at $1/t^2 = 0$ (Hewlett Packard Manual for Model 5901B Auto-viscometer).

A cone-in-cone viscometer to the design of Dintenfass (1963) was used for readings at controlled shear rates from 7·3 sec.$^{-1}$ upwards. Viscosity of a 25 per cent. solution of glycerol in water at 25·5°C. was 1·77 ± 0·19 ($n = 10$) at shear rates between 7·3 and 183 sec.$^{-1}$ compared with an expected 1·80 (Sheely, 1932). Close correspondence was also obtained with hyaluronic acid solutions at similar shear rates in the capillary and cone-in-cone viscometers.

Results from the capillary viscometers were corrected for density, the maximum corrections being about 4 per cent. for concentrated sugar solutions. All results are expressed as viscosity ratios relative to buffer at 25°C., *i.e.* as multiples of about 0·9 centipoises ($\eta_r = 1·0$). The term η will be used to signify η_r unless specified otherwise.

Results

SYNOVIAL FLUID

Stability of viscosity was first assessed with the M2 viscometer in aliquots of a 1:1 (v/v) mixture of synovial fluid and serum. The initial η_r fell by 0·25 per cent. after 3 days at room temperature, by 1·3 per cent. in 7 hrs at 25°C., and by 2·3 per cent. after freezing and thawing. On the whole, losses after freezing and thawing were infrequent and mainly related to small precipitates removed by the preliminary centrifugation.

Sixteen exploratory experiments were done in which synovial fluid was mixed with buffer or with whole serum proteins, albumin, or other additives in final concentrations selected to give viscosity ratios about 1·2 when measured alone in buffer. The hyaluronic acid content of the synovial fluid dilutions ranged from 0·085 to 1·0 mg./ml., and the corresponding viscosity ratios from 17·37 to 1·34. The series of studies was primarily concerned with achieving pH control, but in every case, regardless of efficiency of buffering, the viscosity of the synovial fluid was increased by the additive, and the order of increase in terms of specific increment (*i.e.* $\eta_{sp} = \eta_r - 1$, where buffer viscosity = 1) far exceeded the sum of the specific increments of each component as measured in buffer alone. Examples are given in Tables I to III, where pH was closely matched by previous dialysis against buffer.

The preliminary results thus suggested that mixture of any viscous solution with synovial fluid would lead to a multiplicative rather than an additive change in the total viscosity of the mixture. Moreover, the order of change seemed better related to viscosity of the added solution rather than concentration of its solute.

* η = viscosity, ρ = density, t = time of flow.

Table I *Effect of added serum protein on viscosity of synovial fluid in a range of dilutions*

Viscosity			Concentration		
Serum alone	*Synovial fluid alone*	*Synovial fluid + serum*	*Hyaluronic acid (mg./ml.)*	*Synovial protein (mg./ml.)*	*Serum protein*
1·20	7·03	12·38	0·80	7·0	38
	4·59	7·37	0·59	5·1	38
	3·08	4·52	0·40	3·5	38
	1·82	2·54	0·19	1·7	38
	1·34	1·87	0·10	0·8	38

pH of solutions in range 7·1 to 7·3.

Table III *Effect of other additives on diluted synovial fluid*

Viscosity			
Additive alone	*Synovial fluid alone*	*Synovial fluid + additive*	*Concentration of additive (w/w)*
Glycerol 1·27	5·51	12·44	12·5%
Glucose 1·22	5·51	11·09	8·5%
Sucrose 1·20	5·51	11·84	8·3%
Glycol 1·23	5·51	10·99	1·25%
Ficoll 1·27	5·51	10·89	1·5%

pG of solutions in range 7·0 to 7·2.

Table II *Effect of added glycerol on viscosity of synovial fluid in a range of dilutions*

	Viscosity		Concentration		
Glycerol alone	*Synovial fluid alone*	*Synovial fluid + glycerol*	*Hyaluronic acid (mg./ml.)*	*Synovial protein (mg./ml.)*	*Glycerol (w/w)*
1·27	9·68	13·33	0·8	7·3	12·5%
	3·60	4·97	0·4	3·6	12·5%
	2·17	2·84	0·2	1·8	12·5%

pH of solutions in range 7·2 to 7·25.

PURIFIED HYALURONIC ACID

Initially, sucrose, whole serum proteins, gamma globulin, or albumin,* were mixed in varied concentrations with a fixed concentration of hyaluronic acid (Fig. 1). The same magnifying effect on η_r of the mixtures was seen as with synovial fluid. The order of change was distinctly different for each solute. In terms of the viscosity of the additive alone, the effects of serum proteins and gamma globulin were similar except for one outlying observation, but significantly less than that of sucrose and treater than that of albumin. These findings were next explored in more detail to establish the relationships in terms of both viscosity and concentration.

* These solutes will be referred to as additives.

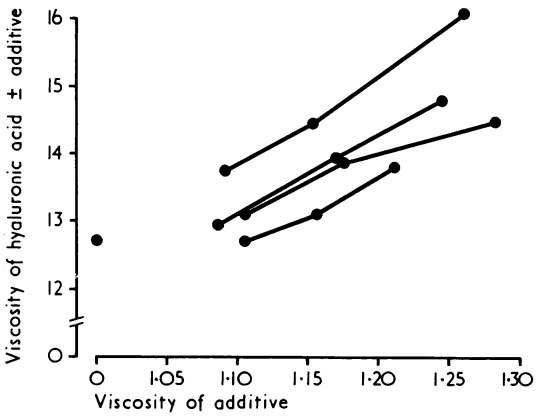

FIG. 1 *Viscosity of solutions of hyaluronic acid with various added solutes, compared with viscosity of those solutes alone. Capillary viscometer. Note that horizontal scale is expanded. Concentration of hyaluronic acid* 1·0 *mg./ml. Single point on left: hyaluronic in buffer alone. Curves, from above downward: hyaluronic acid with sucrose, globulin, serum proteins, or albumin respectively. Coefficients of variation for* η *ranged from* 0·03 *to* 0·46 *per cent.*

The concentrations of the various solutes were varied by halving dilutions both in the pure solutions and in the mixtures and all dilutions were done gravimetrically for greater accuracy. The peak concentration of hyaluronic acid in each experiment was 0·8 mg./ml., and each mixture was diluted with a stock solution of additive, or with buffer alone, so that the concentration of additive was kept constant or reduced in step with that of hyaluronic acid. When the additive was constant, the viscosity of the mixtures consistently exceeded a simple summative relationship from the highest to the lowest concentrations of hyaluronic acid. When both solutes were diluted, the resultant viscosity in each case approached that of hyaluronic acid alone (Table IV, overleaf).

These data were submitted to a closer analysis of the inter-relationships between the effects of the several solutes. Preliminary plotting of the figures for hyaluronic acid alone suggested an empirical functional relationship of the nature $\log_e \eta_H = \alpha_H C_H^{n_H}$ ($\eta_H = \eta_r$ for hyaluronic acid, α_H and n_H are specific

Table IV *Viscosity of purified hyaluronic acid measured alone and with varying or constant concentrations of sucrose or proteins*

(A) Hyaluronic acid (HA) alone					
Concentration (mg./ml.)	0·8	0·4	0·2	0·1	0·05
Viscosity	9·329	3·526	2·021	1·524	1·256
Coefficient of variation (%)	(0·35)	(0·04)	(0·14)	(0·50)	(0·60)
(B) With sucrose					
B1 Sucrose alone					
Concentration (mg./ml.)	82	41	20·5	10	N.D.
Viscosity	1·182	1·087	1·055	1·029	
Coefficient of variation (%)	(0·05)	(0·03)	(0·26)	(0·21)	
B2 H.A. + sucrose (varying)					
Concentration (mg./ml.)	82	41	20·5	10	5
Viscosity	16·574	4·711	2·404	1·609	1·287
Coefficient of variation (%)	(0·27)	(0·15)	(0·15)	(0·14)	(0·26)
B3 H.A. + sucrose (constant)					
Concentration (mg./ml.)	82	82	82	82	82
Viscosity	16·954	5·989	3·349	2·355	1·904
Coefficient of variation (%)	(0·35)	(0·09)	(0·07)	(0·16)	(0·09)
(C) With albumin					
C1 Albumin alone					
Concentration (mg./ml.)	53	26·5	13·3	6·6	3·3
Viscosity	1·245	1·123	1·058	1·028	1·019
Coefficient of variation (%)	(0·43)	(0·36)	(0·33)	(0·11)	(0·25)
C2 H.A. + albumin (varying)					
Concentration (mg./ml.)	0·8 + 53	0·4 + 26·5	0·2 + 13·3	0·1 + 6·6	0·05 + 3·3
Viscosity	14·117	4·459	2·323	1·588	1·272
Coefficient of variation (%)	(0·12)	(0·09)	(0·07)	(0·07)	(0·10)
C3 H.A. + albumin (constant)					
Concentration (mg./ml.)	0·8 + 53	0·4 + 53	0·2 + 53	0·1 + 53	0·05 + 53
Viscosity	14·033	5·200	3·091	2·271	1·913
Coefficient of variation (%)	(0·19)	(0·21)	(0·38)	(0·20)	(0·30)
(D) With serum					
D1 Serum alone					
Concentration (mg./ml.)	31·5	15·75	7·88	3·94	N.D.
Viscosity	1·219	1·102	1·056	1·029	
Coefficient of variation (%)	(0·21)	(0·05)	(0·03)	(0·06)	
D2 H.A. + serum (varying)					
Concentration (mg./ml.)	0·8 + 31·5	0·4 + 15·75	0·2 + 7·88	0·1 + 3·9	0·05 + 2
Viscosity	14·199	4·461	2·355	1·583	1·268
Coefficient of variation (%)	(0·42)	(0·28)	(0·13)	(0·18)	(0·12)
D3 H.A. + serum (constant)					
Concentration (mg./ml.)	0·8 + 31·5	0·4 + 31·5	0·2 + 31·5	0·1 + 31·5	N.D.
Viscosity	14·914	5·198	2·998	2·219	
Coefficient of variation (%)	(0·06)	(0·15)	(0·35)	(0·05)	
(E) With gamma globulin					
E1 Gamma globulin alone					
Concentration (mg./ml.)	32·5	16·25	8	4	N.D.
Viscosity	1·250	1·116	1·054	1·031	
Coefficient of variation (%)	(0·35)	(0·35)	(0·09)	(0·11)	
E2 H.A. + gamma globulin (varying)					
Concentration (mg./ml.)	0·8 + 32·5	0·4 + 16·25	0·2 + 8	0·1 + 4	0·05 + 2
Viscosity	15·308	4·649	2·378	1·603	1·278
Coefficient of variation (%)	(0·31)	(0·27)	(0·07)	(0·06)	(0·13)
E3 H.A. + gamma globulin (constant)					
Concentration (mg./ml)	0·8 + 32·5	0·4 + 32·5	0·2 + 32·5	0·1 + 32·5	0·05 + 32·5
Viscosity	15·343	5·608	3·262	2·371	1·982
Coefficient of variation (%)	(0·30)	(0·30)	(0·36)	(0·31)	(0·40)

constants, and C_H = concentration of hyaluronic acid). Similar relationships were determined for the additives, and the simplest multiplicative hypothesis for predicting the viscosity of a mixture was tested according to the formula:

$$\log_e \eta_{H+a} = \alpha_H c_H^{n_H} + \alpha_a c_a^{n_a}$$

(Subscript *a* refers to the additive).

This was found to underestimate the observed results. To obtain a better fit within a model of this type, a correction factor with one further characteristic was introduced in the following form:

$$\log_e \eta_{H+a} = \alpha_H c_H^{n_H} + \alpha_a c_a^{n_a} + \alpha_{H_a} c_H^{n_H} c_a^{n_a}.$$

This model was fitted to all the data, using the method of least squares to compute the various coefficients. These are shown in Table V.

Table V *Coefficients for relationships between viscosity and concentration of solutes** ($\eta = e^{\alpha c_n}$, etc. See text)

Solutes	α_H	α_a	α_{Ha}	n_H	n_a
Hyaluronic acid (H)	2·604			0·7713	
+ sucrose (*a*)	2·604	0·0033	0·0052	0·7713	1·005
+ albumin (*a*)	2·604	0·0061	0·0038	0·7713	0·980
+ serum proteins (*a*)	2·604	0·0086	0·0106	0·7713	0·965
+ gamma globulin (*a*)	2·604	0·0082	0·0067	0·7713	1·055

* mg./ml.

Expected values for viscosity were derived from these formulae, and agreed well with observed values as shown in Fig. 2.

NON-NEWTONIAN VISCOSITY

Hyaluronic acid, as it occurs in synovial fluid, shows a pseudoplastic type of non-Newtonian viscosity; that is, its viscosity increases as rate of shear falls. In capillary flow the shear rate, dv/dr, is inversely related to viscosity,* and the increases in viscosity observed above might therefore have been exaggerated by a decrease in shear, though the lowest shear rate would have been at least 75 sec.$^{-1}$ (*e.g.* 1300/7, Expt. 1 in Table I). Mixtures of hyaluronic acid with a variety of additives were therefore studied in the cone-in-cone viscometer (Fig. 3). Small deflections of this instrument's torsion wire were difficult to read accurately. Multiple readings for buffer were therefore standardized by regression analysis constrained to zero rate of shear, and the viscosity ratios were expressed as a factor of this regression. Estimates of the additives gained from capillary studies were used for comparisons in Fig. 3 (overleaf).

* $\frac{dv}{dr} = \frac{-Pr}{2\eta l}$, where v = linear velocity, P = pressure head, r = radius, and l = length of capillary.

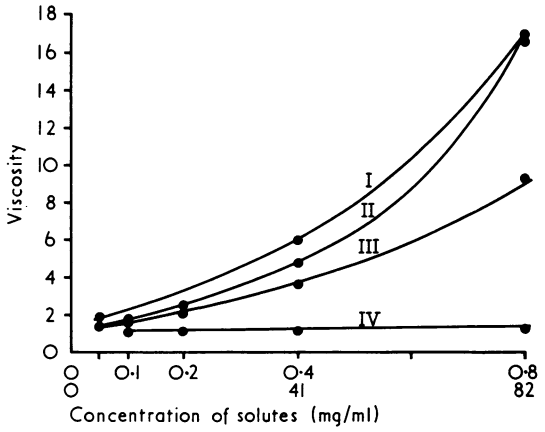

FIG. 2 *Viscosity of hyaluronic acid alone and with sucrose. Comparison of observed and predicted values as described in text. Points indicate observed values, continuous lines predicted values.*

Ordinate: viscosity ratio (η_r).

Abscissa: concentrations. Upper, hyaluronic acid. Lower, sucrose.

(I) Hyaluronic acid + fixed concentration of sucrose (82 mg./ml.).

(II) Hyaluronic acid + varied concentration of sucrose

(III) Hyaluronic acid alone.

(IV) Sucrose alone.

Three conclusions can be drawn:

(1) The additives increased the viscosity of hyaluronic acid in a synergistic manner throughout the range of shear rate examined;

(2) Serum proteins caused a lesser increase in viscosity than the non-ionic additives with a similar range of viscosity;

(3) The factorial increase in viscosity tended to remain at least the same throughout the range of shear rate, and appeared to increase further at low shear rates with those additives that gave the greatest increase in total viscosity. η_r for most of the mixtures, except at the extremes of shear rate for serum M, fell outside 1 per cent. confidence limits for the regression of $\log_e \eta_H$ against $\log_e$ sec.$^{-1}$.

Discussion

The relationships between viscosity and concentration were empirically derived. In the case of hyaluronic acid the form is similar to that given by Ropes and others (1947) and by Ragan and Meyer (1949). Different forms might be chosen for the saccharides and proteins, but would not materially alter the interpretation of their interactions with hyaluronic acid.

The total viscosity of two classes of solute behaving ideally in a single solution might be expected to be

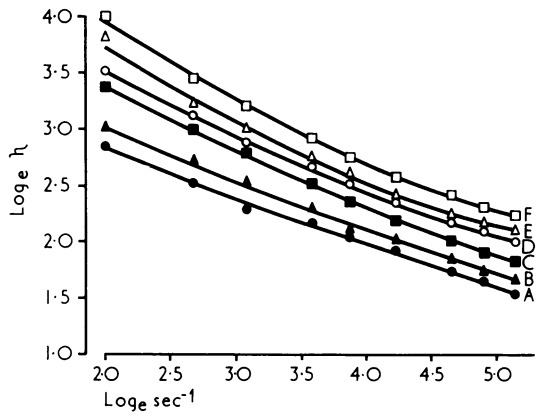

FIG. 3 *Effect of added solutes upon viscosity of hyaluronic acid at controlled rates of shear (sec.$^{-1}$).*

A: Hyaluronic acid alone:

Viscosities of additives alone were:

B: Hyaluronic acid + serum M serum M, η 1·27, log$_e$ = 0·2390

C: Hyaluronic acid + serum G serum G; η 1·23, log$_e$ = 0·2070

D: Hyaluronic acid + sucrose sucrose, η 1·20, log$_e$ = 0·1823

E: Hyaluronic acid + glucose glucose, η 1·22, log$_e$ = 0·1989

F: Hyaluronic acid + glycerol glycerol, η 1·27, log$_e$ = 0·2390

Addition of the logarithms of η_r for each solute in the mixed solutions to the figures for hyaluronic acid alone shows that the resultant viscosities at all rates of shear exceeded the products of the viscosities of the constituents, except in the case of serum M.

derived from the product rather than the sum of their individual concentrations, or of their viscosities measured separately, but the possible physical and chemical interactions between hyaluronic acid and proteins do not allow prediction of the direction or degree of change in viscosity resulting from the mixture.

The serum proteins, albumin and gamma globulin, which are not associated with hyaluronic acid in ultrafilter residues of synovial fluid (Curtain, 1955), can clearly cause large increases in viscosity when mixed with hyaluronic acid and do so in concentrations commonly found in joints. The relationships given here are consistent in form for two classes of solute, *i.e.* sucrose and protein, which differ greatly in molecular weight and conformation, ionic structure, and propensity for formation of different types of physical and chemical bond. The general nature of the relationships would therefore seem a valid basis for analysing the viscous behaviour of hyaluronic acid. Some variation in the constants should be expected with different samples, although the formula for hyaluronic acid in Table IV gave a value within 7 per cent. of the estimated value for the batch of hyaluronic acid used in the previous experiment.

Although the highest concentration of hyaluronic acid in this study was rather lower than that of normal human synovial fluid, it was well within the range found in synovial effusions, and the protein concentrations covered the whole of the normal (13–20 mg./ml.) and part of the abnormal range (up to 60 mg./ml.) in synovial fluid. The increases in viscosity bore a direct multiplicative relationship to the concentrations of the hyaluronic acid and protein or other added solute as expressed in the formulae given earlier. Furthermore, an additional magnifying term was required to gain a full estimate of viscosity in the presence of each added solute, whether sucrose or proteins. The magnitude of the first coefficient, α_a, can be related to molecular weight of the solute, but there is a distinction in this respect between sucrose and the proteins in the second coefficient, α_{Ha}.

Thus, the potential contribution of protein to the viscosity of synovial fluid can be grossly underestimated if it is considered in terms of its viscosity alone. The relationships found in this study indicate that intrinsic viscosity of hyaluronic acid can be safely derived from whole or fractionated synovial fluid if the protein is sufficiently diluted before final extrapolation to zero concentration. However, intrinsic viscosity, by definition, cannot measure the contribution of protein to the viscosity of synovial fluid in real concentrations although it is a valid molecular parameter of hyaluronic acid.

In seeking to explain the physical differences between normal and rheumatoid synovial fluids, Ferguson, Boyle, and Nuki (1969) found that urea and guanidine hydrochloride greatly reduced viscosity of osteoarthritic but not of rheumatoid synovial fluid, which suggests that a macromolecular complex exists in the former but not the latter. However, these agents must affect the colligative properties of all constituents of aqueous solutions and might have different effects in the two classes of fluid simply because there are different ratios of protein to hyaluronic acid and different proportions of the subclasses of protein in each type of fluid. The term 'complex' implies a molecular association with or without obvious coacervation or phase separation and raises questions of selective bonds of physical or chemical nature. We wish merely to draw attention to the nature of the gross changes in viscosity without speculating upon their mechanism at this stage.

The techniques used in this study do not permit any inferences about changes in elastic properties of synovial fluid (Ogston and Stanier, 1953; Balazs and Gibbs, 1970) which require different methods. Nevertheless, increases in viscosity would independently impose increased work loads during a given movement or flow (Yang, 1961).

The viscous interaction of hyaluronic acid and proteins may be relevant to several aspects of synovial function. In synovial effusions, the concentration of protein and the relative proportion of globulins both increase, which would tend to lessen the drop in viscosity due to concurrent dilution or to lesser polymerization of the hyaluronic acid. The role of viscosity in lubrication of joints is uncertain at present. Hyaluronic acid seems to be more important in the lubrication of synovial membrane than of cartilage (Radin, Paul, Swann, and Schottstaedt, 1971). Increases in viscosity would, as noted above, increase the work-load of a joint especially in the periarticular tissues where the rate of shear is likely to be lower. The viscous reaction of protein with hyaluronic acid would thus contribute to 'morning stiffness' through inflammatory exudation and increase in the concentration of proteins in the surrounding connective tissue. This would be more pronounced if there is a temporary accumulation of exuded protein as a result of reduced lymph flow with overnight inactivity. Since diffusion is directly related to viscosity, inflammatory exudation of proteins into connective tissue ground substance would also enhance the hindrance to diffusion presented by hyaluronic acid.

Summary

(1) Hyaluronic acid was freed from protein by ultra-centrifugation of synovial fluid in caesium chloride.

(2) The viscosity of solutions of this hyaluronic acid was found to bear an exponential relation to its concentration in the range 0·05 to 0·8 mg./ml.

(3) The resultant viscosity of mixtures of hyaluronic acid with sucrose, total serum proteins, serum albumin, or gamma globulin exceeded values calculated from the product of the viscosities of each component measured alone in equivalent concentration, or the product of the exponential functions relating the viscosity to the concentration of each. This multiplicative effect of mixed solutes on total viscosity of hyaluronic acid is at least partly independent of rates of shear.

(4) Similar results were consistently found in studies with unfractionated synovial fluid and added viscous solutes.

(5) It is concluded that major proteins of synovial fluid, which are not known to form complexes with hyaluronic acid under physiological ionic conditions, can make a substantial contribution to the total viscosity of the fluid. The possible significance of this viscous interaction in inflamed joints and connective tissue is discussed.

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