2. An empirical correction for the effect of albumin on the sedimentation of hyaluronic acid is obtained, and applied to the results obtained for human synovial fluids.

3. The effect of albumin on the sedimentation of hyaluronic acid, when the latter is the slower component, is shown to be consistent with the 'McFarlane' (1935*a*, *b*) boundary anomaly.

4. The effect of albumin on the sedimentation of hyaluronic acid and the reverse effect, in regions of the cell where both are present, have been investigated. The former is shown to be consistent in general with current theory. The latter effect requires special explanation, and the hypothesis is put forward that a large fraction of the hyaluronic acid particle is not penetrable by albumin.

The author is most grateful to Dr A. G. Ogston for encouragement and for very helpful discussions; to Mr R. G. Taylor of the Wingfield-Morris Orthopaedic Hospital, Oxford and to Dr Robb-Smith of the Radcliffe Infirmary, Oxford, for supplies of human synovial fluid, and to the Oxford Co-operative Society Ltd., for facilities at their Botley Road slaughterhouse.

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A Comparison of the Properties of Hyaluronic Acid from Normal and Pathological Human Synovial Fluids

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A number of studies have previously been carried out on synovial fluid from both human and animal sources. The present work was undertaken primarily to compare the hyaluronic acid in fluids from normal and pathological human knee joints. Because of the small volume of fluid available from a normal joint, investigation by electrophoresis or by preparation of ultrafilter-residue (Ogston & Stanier, 1950) was precluded and these methods were accordingly not used on pathological effusions. The methods used included ultracentrifugal and viscosity measurements, the formation of mucin, and the study of its composition. It was hoped that any difference between these properties in normal and pathological fluids might throw light on any changes in the hyaluronic acid in trauma and disease. Furthermore, an assessment of the results from these techniques might reveal a reliable and sensitive test for abnormality in joint fluids.

EXPERIMENTAL

Synovial fluids, sources and collection

Knee-joint fluid from freshly slaughtered cattle was collected and stored as described by Ogston & Stanier (1950). Human synovial fluid from knee joints was obtained with full sterile precautions. The human samples, may be divided into three groups: (i) fluids from normal joints, (ii) fluids from joints as near normal as possible, and (iii) frankly pathological fluids. Those in the intermediate group were obtained only from joints which appeared at operation to be nearly normal; all injuries were past the acute phase and the joints had been previously rested. Brief data on the samples are given below.

Normal group. *A* and *B*: normal joint fluid obtained at operation, 1 ml. *J*: post-mortem sample obtained 12 hr. after death, 1 ml.

Intermediate group. *C*: internal derangement of the knee, approx. 15 ml. *D*: pooled sample from four cases of cartilage operation, approx. 12 ml. *E*: cartilage operation. Slightly blood-stained, 8 ml. *F*: cartilage operation, 3 ml. *G*: cartilage operation, 8 ml.

Pathological group. *H*: acute trauma with large effusion, approx. 40 ml. *K* and *L*: untreated case of rheumatoid arthritis. Fluids from right and left joints respectively.

M and *N*: from the same joints as *K* and *L* after treatment with cortisone. Samples *K*–*N* were obtained by partial aspiration of large effusions.

Storage. All fluids were cooled to 4° as soon as possible. Human fluids retained their viscosity at this temperature for from 3 weeks to several months, despite brief removal to room temperature and contamination on withdrawal of part of the sample. After 12 hr. at room temperature a fluid fell in viscosity and therefore during dialysis or prolonged experiments all fluids were kept at 4°, but no sterile precautions were taken for brief experiments. Fluids were

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dialysed against a standard buffer before use (0·2 M-NaCl, 0·0077 M-Na_2HPO_4, 0·0023 M-KH_2PO_4, pH 7·3).

Certain fluids during the first few days of storage in the cold, threw down a voluminous clot. One of these clots was removed, washed and redissolved in M-Na_2CO_3. This solution gave positive tests for protein and carbohydrate. In the ultracentrifuge it proved heterogeneous, but showed a diffuse component of S_{20} 1·5. Since the viscosity of a fluid was unaffected by the appearance of a clot, fluids were centrifuged before use and any clot rejected.

Methods

Ultracentrifuge. A Svedberg oil-turbine ultracentrifuge with a diagonal schlieren optical system (Philpot, 1938) was used. All running procedure and sedimentation constant calculations were carried out as described by Cecil & Ogston (1948).

Viscosity. Routine viscosity measurements were made in an Ostwald viscosimeter (capacity 0·95 ml. and water-flow time of about 24 sec.). Studies on viscosity at different velocity gradients were carried out in a co-axial cylinder Couette-type viscosimeter (Ogston & Stanier, 1953*a*). This had a capacity of 10 ml. of fluid and a range of velocity gradient up to 75 $sec.^{-1}$. All viscosity measurements were made at 25°.

Total nitrogen. Total nitrogen was estimated by ashing and nesslerization. The colour developed was measured in a Hilger 'Biochem' photoelectric colorimeter.

Glucosamine. Glucosamine in mucin was estimated by the method of Johnston, Ogston & Stanier (1951).

Refractive increment. Two refractometers were used to measure refractive increment. The first was a double-prism refractometer (designed by Dr J. St L. Philpot); the second was a modification of this (Cecil & Ogston, 1951). Light of 546 and 578 mμ., respectively, was used in the two refractometers. Determinations were accurate to 2×10^{-6} of refractive index (S.D.).

Mucin preparation. Mucin clots were prepared by the small scale method of Ogston & Stanier (1950).

Dry weight. The dry weight of mucin was measured by heating to constant weight at 105°.

Estimation of hyaluronic acid concentration. An immediate problem arose as to the method chosen to estimate hyaluronic acid concentration. Provisionally concentration was referred to dry weight of mucin. All fluids examined gave a satisfactory clot in a clear solution, although the friability of the clot varied. Two methods were employed. In the first, mucin clots were prepared from a known volume of fluid after dialysis, and heated to constant weight. In some cases mucin clot formation after dialysis was unsatisfactory; the original fluids were therefore used and errors from volume change on dialysis eliminated by quantitative transfer of the sac contents with washing after dialysis was complete.

The second method depended on refractive increment estimation. A calibration curve was prepared for the alteration in the refractive increment of buffer on dilution with small quantities of distilled water. Pairs of mucin clots from the same fluid were prepared, one of each pair was heated to constant weight. The second was added to a weighed quantity of buffer, and the resulting solution weighed and its refractive increment estimated against that of buffer. The weights of the wet and dry clots gave the weights of water and mucin added to the buffer and from these the specific refractive increment of mucin could be calculated. The mean of five estimations gave a result of 0·00164 ± 0·00004 (S.D.).

Refractive increment estimations could now be used to measure mucin concentration. A single clot was prepared, weighed, redissolved in buffer, and the refractive increment of the solution measured as described above. As a first approximation the buffer was assumed to have been diluted by water corresponding to the wet weight of the clot, the refractometric measurement then allowed the true dry weight of the clot to be obtained by successive approximation. This method was more rapid, and was of value with small fluid samples since it left a mucin solution for further experiments.

RESULTS

Composition of mucin. The total nitrogen and glucosamine contents of mucin prepared from five different fluids were examined. Total nitrogen estimations gave a value of 11·1% ± 0·8 (S.D.). Glucosamine estimations gave a value of 11·9% ± 0·6 (S.D.). All the fluids in this experiment formed satisfactory clots although the clinical normality of the joints varied considerably. There was no correlation between these analytical figures and the normality of the fluid as indicated by other tests. These mean values and that of the specific refractive increment given above agreed satisfactorily with previous estimations on human fluids and with those obtained from studies on ox fluid (Ropes, Robertson, Rossmeisl, Peabody & Bauer, 1947; Ogston & Stanier, 1952, 1953*b*). In view of these results and the uncertain importance of mucin no further analyses were made.

Ultracentrifuge results

General. Forty-six ultracentrifuge runs were carried out on hyaluronic acid and its derivatives. Fig. 1 gives typical sedimentation schlieren

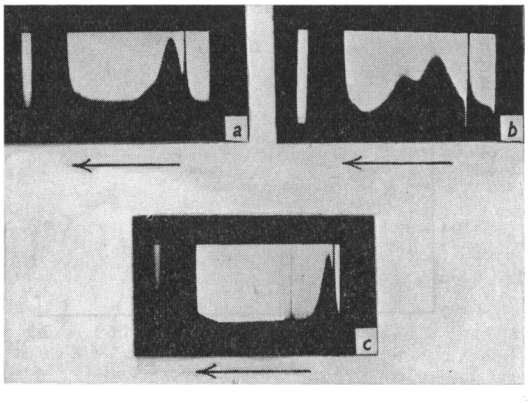

Fig. 1. Ultracentrifuge diagrams of (*a*) fluid from normal group, (*b*) fluid from rheumatoid arthritis group, (*c*) fluid from intermediate group showing the presence of a fast component. The arrows indicate the direction of sedimentation.

diagrams for a normal human fluid, for a pathological fluid, and for a fluid of the intermediate group showing a fast component. The rate of sedimentation of the hyaluronic acid was affected by the presence of protein. This effect is due to a boundary anomaly operating in the sedimentation of mixtures and not to an effect of protein on the molecular properties of hyaluronic acid (Johnston, 1955*a*). The protein concentration of each solution was therefore estimated by integration of the sedimentation diagrams and all values for the S_{20} of hyaluronic acid were corrected for the presence of protein, being referred to hyaluronic acid sedimenting alone. Fig. 2 shows a plot of the reciprocal of these corrected S_{20} values against the log of the Ostwald viscosity of the solutions. The hyaluronic acid from different sources shows marked variation in its properties. The reality and significance of these differences will be discussed later.

Fast components. In seven of the fluids there was an additional fast-moving boundary (Fig. 1*c*). The times of origin of these were found by extrapolation to be approximately the same as those of the other components. This showed that they represented a genuine sedimenting component and were not a convection artifact. In fourteen cases the sedimentation constants were measured. Since the fast boundaries only persisted for from 5 to 40 min. after reaching full speed, accurate determinations were not always possible. (Measurement was limited in time and had to start immediately on nearing full speed when rotor temperature and speed were still changing.) The sedimentation constants varied between 9·1 and 15·2. They tended to rise in dilute solution and the values at infinite dilution lay between 17 and 20. No marked correlation existed between S_{20} and the viscosity of the solution, the concentrations of hyaluronic acid or albumin, or the time of persistence of the boundary. This last was chosen as a possible means of estimating the concentration of the fast component.

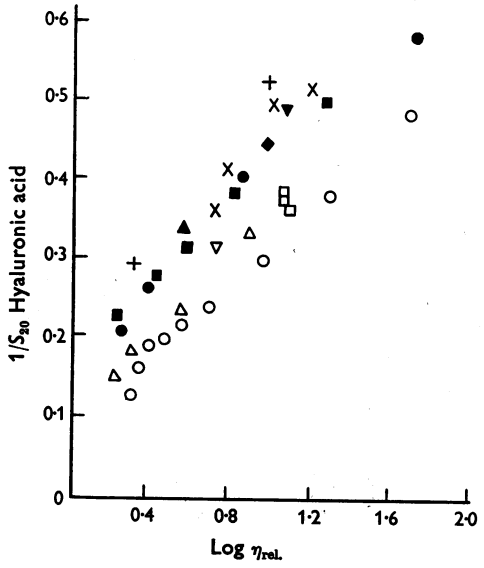

Fig. 2. Variation in $1/S_{20}$ of hyaluronic acid with log viscosity of the solution. Normal group of fluids: △, sample *A*; ▽, sample *B*; ○, sample *J*; □, ox fluid. Intermediate group of fluids: ■, sample *C*; ●, sample *D*; ▲, sample *E*; ▼, sample *F*; ◆, sample *G*. Pathological group of fluids: +, sample *H*; ×, samples *K*–*N*.

It is likely that these fast components arose from hyaluronic acid. The boundaries resembled those of hyaluronic acid and in two cases appeared only in dilutions of the original fluid. In a run on an accidentally infected fluid the hyaluronic acid boundary appeared reduced and there were two fast-moving boundaries identical with those of fast component. The S_{20} values of the fast boundaries were, however, 16·3 and 27·0.

The formation of fast component on dilution of a synovial fluid was suggested by the following: (1) in two cases no fast component appeared in the original solution; (2) where fast components were present in more than one of the dilutions of a fluid, the concentration of the fast boundary appeared to remain constant or to increase in the more dilute solutions. (This observation was based both on the appearance of the boundary and on its time of persistence.) Even where the concentration of the boundary appeared constant on serial dilution, this would indicate a greater proportion of the hyaluronic acid appearing in this form. The possibility that this was due to a change progressing with time rather than with dilution was eliminated in the case of sample *C*. Runs were performed on three dilutions (all showing fast components) before the original fluid was ultracentrifuged. The original fluid showed no fast component.

If these boundaries arise from hyaluronic acid, then, whatever the reason for their appearance, they must represent a form of hyaluronic acid sufficiently homogeneous to give a single ultracentrifuge boundary. This may not entail a high degree of homogeneity, since the variation of S_{20} with concentration must produce a considerable 'sharpening' effect on the boundaries of hyaluronic acid. (This is illustrated by sample *D* which, though a pooled sample, formed a single boundary.) The variation of S_{20} with concentration appears less in the case of the fast component but this is difficult to assess, since concentration estimation is uncertain. A marked sharpening effect may still operate with the fast component and cause a heterogeneous component to form a single boundary. If the increase in S_{20} represents an alteration in the size or form of the hyaluronic acid molecule giving rise to a range of compounds, this sharpening will explain

the appearance in different synovial fluids of single boundaries with differing sedimentation properties. The S_{20} observed will represent an average for the range of compounds dependent on the proportions present.

Albumin/globulin (A/G) ratios. The A/G ratios were estimated from the sedimentation diagrams. Resolution of the peaks was carried out by the methods described in Johnston & Ogston (1946). Satisfactory agreement was found between different diagrams in the same run except where the ratio was above 10. These values do not represent the true A/G ratio because of the McFarlane effect and because some of the globulins of plasma may sediment in the ultracentrifuge with the albumin. An approximate correction for the former was made; the presence of the hyaluronic acid was ignored and a correction based on the equation for the concentration in the trailing boundary of a sedimenting mixture was applied (Johnston & Ogston, 1946). This correction proved small and was only used where the total protein present exceeded 2%. The A/G ratios so obtained are given in Table 1. The values for the 'normal' human fluids are higher than those for ox fluids. A rheumatoid arthritis patient after treatment with cortisone showed a slight increase in the A/G ratio of the fluids of both knee joints (samples *M*, *N* compared with *K*, *L*).

Table 1. *The albumin/globulin ratios and intrinsic viscosities for different human synovial fluids*

Intrinsic viscosity is $(\eta_{rel.}-1)/\phi$, extrapolated to $\phi=0$, where ϕ is the hydrated volume fraction.

Sample	Albumin/globulin ratio	Log intrinsic viscosity
A	17·0	4·20
C	7·6	3·55
D	9·5	3·10
E	9·3	3·25
F	7·8	3·25
G	10·0	3·25
H	3·6	3·70
J	14·0	3·80
K	0·83	—
L	1·6	—
M	1·4	—
N	2·4	—

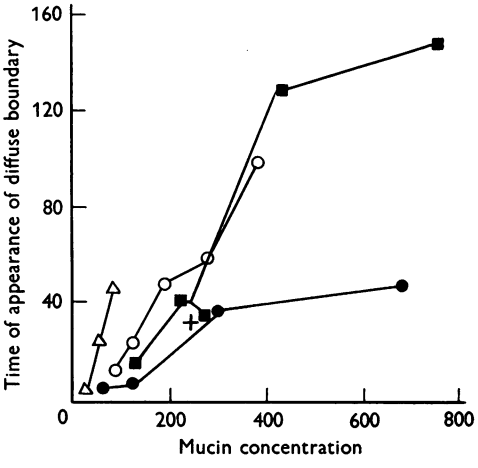

Fig. 3. Variation in the time of appearance of a diffuse boundary during sedimentation with the concentration of the hyaluronic acid boundary. (Time expressed as minutes after reaching full speed, hyaluronic acid concentration as mg. mucin/100 ml. in the original fluid.) The symbols refer to different samples of synovial fluid as in Fig. 2.

Diffuseness of the boundary. In ultracentrifuge experiments the hyaluronic acid boundary at first appeared very sharp but in some cases became diffuse during the course of sedimentation. This change showed as a rapid blurring or disappearance of the boundary 'spike' with a simultaneous broadening of its base (Ogston & Stanier, 1950). For any given sample of fluid the time taken for this to happen was roughtly proportional to the hyaluronic acid concentration (Fig. 3).

The behaviour, however, of different samples varied considerably. For the same concentration of hyaluronic acid, the normal fluids took longer to produce a diffuse boundary than did those of the other groups. (In view of the rough nature of this test the value of the hyaluronic acid concentration in the original fluid was used and no attempt made to correct for concentration effects when hyaluronic acid formed the trailing boundary. Since the more abnormal fluids tended to have a higher protein content, this correction, if it were applied, would increase the differences found by this test.) An apparent exception to the observation that normal fluids took longer to form a diffuse boundary, was sample *D* which, though it belonged to the intermediate group, had the greatest tendency to produce a diffuse boundary. This sample was obtained by pooling four separate fluids and the diffuseness was probably due to heterogeneity of the hyaluronic acid from these different sources.

Viscosity. Viscosity measurements by Ostwald viscosimeter were made on all fluids and their dilutions. There was considerable variation in the relationship between the mucin content and the Ostwald viscosity of different samples (Fig. 4).

In three cases sufficient fluid was available for measurement in the Couette viscosimeter, and the viscosity of serial dilutions was measured. Values for $\eta_{rel.}$ for zero velocity gradient were obtained by extrapolation of a plot of $1/\eta_{rel.}$ against velocity gradient (r); values for infinite velocity gradient were also obtained by extrapolation ($\eta_{rel.}$ against $1/r$). From mucin clot formation, and assuming a value of 45% for the hyaluronic acid in mucin (Ogston & Stanier, 1952) the hydrated volume

fraction (ϕ) of hyaluronic acid could be calculated for each solution. The viscosity expressed as $\log[(\eta_{rel.}-1)/\phi]$ was calculated and a typical plot of this against ϕ is given in Fig. 5. The relative viscosity values obtained from the Ostwald viscosimeter are also included. In some dilutions they show a viscosity less than the so-called 'infinite velocity gradient' values for the Couette viscosimeter, indicating either an error due to capillary flow of a fluid showing anomalous viscosity or to an unsatisfactory extrapolation to obtain $\eta_{rel.(\infty)}$ Couette. Ostwald viscosity always decreased relative to $\eta_{rel.(\infty)}$ Couette on dilution of the fluid. The intrinsic viscosity is independent of the velocity gradient, and the intrinsic viscosity obtained from the Ostwald measurements agrees satisfactorily with the Couette value. This was found in the two other fluids and Ostwald viscosities were used to measure the intrinsic viscosity of samples too small for the Couette viscosimeter (Table 1).

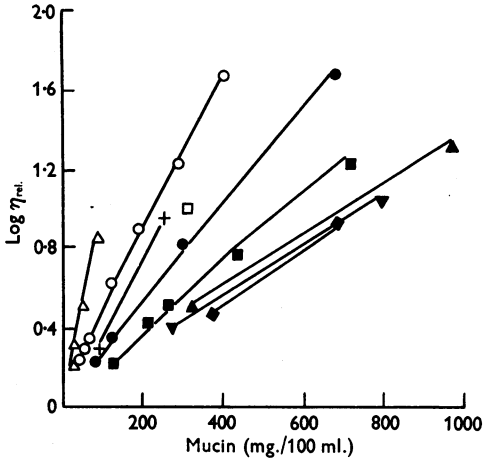

Fig. 4. Variation in the Ostwald viscosity of synovial fluid with the hyaluronic acid concentration estimated as mg. mucin/100 ml. solution. The symbols denote different samples of fluid, as in Fig. 2.

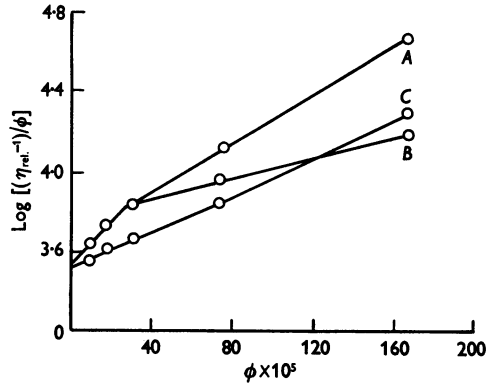

Fig. 5. Variation of $\log[(\eta_{rel.}-1)/\phi]$ with hydrated volume fraction (ϕ) at different velocity gradients. Curve *A*, zero velocity gradient, Couette viscosimeter; curve *B*, infinite velocity gradient, Couette viscosimeter; curve *C*, Ostwald viscosimeter.

Non-Newtonian variation of viscosity with velocity gradient was found in the fluids studied in the Couette viscosimeter, although in some cases dilution removed all detectable variation. The intrinsic viscosity and the degree of the non-Newtonian viscosity ('anomaly') varied between the different samples. This variation in the anomaly might indicate the normality of the fluid. Table 2 shows the intrinsic viscosities of the fluids and the extent of their anomaly. This latter was measured in two ways: (1) the extent of the anomalous viscosity (expressed as

$$\log[(\eta_{rel.(0)}-1)/\phi]-\log[(\eta_{rel.(\infty)}-1)/\phi])$$

for an arbitrarily chosen concentration value ($\phi = 6\times10^{-4}$); (2) the extent of the anomalous viscosity (expressed as $\eta_{rel.(0)}-\eta_{rel.(\infty)}$) compared to $\eta_{rel.(\infty)}$. (The three fluids happened to provide dilutions of approximately the same $\eta_{rel.(\infty)}$ value.) Normal fluids might be expected to have a high intrinsic viscosity and a large anomaly relative to concentration and to viscosity at infinite velocity gradient. The methods of comparison used in Table 2 do not give consistent results for these tests, and this will be discussed below.

Molecular dimensions. As in ox fluid, the intrinsic viscosity was independent of velocity gradient, indicating a nearly spherical molecule. To account for anomalous viscosity at finite concentrations a very voluminous particle must be assumed. From the intrinsic viscosity, S_{20} at infinite dilution and the rate of change of $1/S_{20}$ with concentration [d(1/*S*)/dc] the molecular weight, hydrodynamic specific volume and axial ratio were calculated for some of the samples. The method used was that of Fessler & Ogston (1951) as modified by Ogston (1953). The partial specific volume was taken as 0·69 and hyaluronic acid was assumed to form 45 % of mucin (Ogston & Stanier, 1952). The values obtained are given in Table 3. These estimates can, at best, be considered semi-quantitative. The hydrodynamic theory of the variation of $1/S_{20}$ is imperfect and the

Table 2. *Comparison of the non-Newtonian viscosity of three synovial fluids with the intrinsic viscosity and the infinite velocity gradient viscosity*

Values of η expressed as relative viscosities; subscripts refer to velocity gradients.

Sample	η_∞	$\eta_0-\eta_\infty$	Log_{10} intrinsic viscosity	$\text{Log}_{10}[(\eta_0-1)/\phi]-\log_{10}[(\eta_\infty-1)/\phi]$ $\phi=6\times10^{-4}$
C	7·4	4·3	3·6	0·13
D	9·0	5·0	3·1	0·23
H	9·5	3·1	3·7	0·14

Table 3. *Values of the axial ratio (J), effective hydrodynamic volume (V') and molecular weight (M) of human hyaluronic acids.*

Sample	J	V'	M
A	20	500	$8{\cdot}4 \times 10^6$
J	15	275	$2{\cdot}1 \times 10^7$
C	25	40	$1{\cdot}2 \times 10^6$
D	7	110	$1{\cdot}5 \times 10^6$
H	10	250	$1{\cdot}3 \times 10^6$

value of certain constants used uncertain. They confirm, however, the model of a very voluminous particle of no great asymmetry with a normal molecular weight (M) of the order of 10^7. The method is more accurate in the estimation of molecular weights, and the decrease in M in the abnormal fluids probably indicates a depolymerization of the molecule.

DISCUSSION

Mucin properties. The glucosamine content, total nitrogen and specific refractive increment of mucin obtained from different synovial fluids agree well between themselves and with those for ox fluid. These estimations can only be made on normal and near-normal fluids (those capable of forming clots) and so are of limited application. They show little or no change with the degree of abnormality of the fluid as judged by other tests.

Ultracentrifugal results. The comparison of $1/S_{20}$ for hyaluronic acid with log viscosity of solution showed a large variation between different samples of synovial fluid. This, though reduced, remained after a correction was applied for the protein present (Fig. 2). This residual variation might be due to an inadequate or inaccurate correction for the presence of protein, especially as the value of S_{20} at infinite dilution varied little between samples. (Extrapolation to infinite dilution of hyaluronic acid is also equivalent to extrapolation to zero concentration of protein and might be expected to give a correct S_{20} at infinite dilution even though the correction for the presence of protein was inaccurate.) It is likely, however, that this remaining variation is too large to be accounted for by errors in the correction; and furthermore, in the case of three fluids, an increase in the correction would lead to an even larger variation between different fluids. Agreement between the behaviour of different fluids cannot be obtained by choosing any arbitrary correction to the S_{20} of hyaluronic acid proportional to protein concentration.

If abnormality of hyaluronic acid leads to a decrease in viscosity and an increase or little change in S_{20}, then this residual variation indicates that in abnormal fluids the viscosity and the S_{20} of hyaluronic acid are affected to different extents. Either the viscosity is reduced before the S_{20} value is affected, or else, if they are affected simultaneously, then the fall in viscosity is relatively greater than the rise in S_{20}.

From the time of first appearance of diffuseness in hyaluronic acid boundaries and from the behaviour of the fast component, it was suggested that a single hyaluronic acid boundary in the ultracentrifuge might represent a mixture of hyaluronic acid particles of different size and properties. The variation in the sedimentation behaviour of fluids in the normal group might be explained by this hypothesis, i.e. that hyaluronic acid represents a range of particles and not a particular molecular configuration of fixed ultracentrifugal and viscous properties. According to this the hyaluronic acid of normal synovial fluids will vary in physical properties but will tend to contain a higher proportion of particles having low S_{20} and high viscosity. If this is true, then the fact that the mucin formed from different fluids is constant in its glucosamine and total N contents could be due to either of two causes: (1) that within the normal and near-normal range of molecular size of hyaluronic acid, the weight of protein associated with hyaluronic acid in mucin is dependent more on the quantity of 'hyaluronic acid chain' than on the number of molecules; (2) that a decrease in the size of the hyaluronic acid molecule need only be very small to affect its physicochemical properties, and that in this narrow range the change may be too small to be detected by analytical procedures.

Viscosity measurements. Hyaluronic acid must have nearly spherical molecules with considerable hydration since in the Couette viscosimeter experiments, the intrinsic viscosity was independent of velocity gradient. The fluids examined in the Couette viscosimeter included one from the pathological group (sample H) and the range of intrinsic viscosities obtained by extrapolation of data at different velocity gradients lay between 1260 and 5100, suggesting that no great increase in axial ratio occurs in the hyaluronic acid of pathological fluids.

General. Several of the investigations showed differences in the properties of the various samples of hyaluronic acid. The normality of the hyaluronic acid as judged by ten criteria is roughly indicated in Table 4.

The tests chosen were: (1) Clinical state of the joint. (2) The quantity of fluid present in the joint, the less fluid found the more normal it was presumed to be. (3) The ability to form mucin clots: designated (*a*) normal clot, (*b*) a clot formed in a clear solution but with poor retraction and increased friability, and (*c*) no clear solution obtained or very friable clot. (4) Diffuseness of the boundary in the ultracentrifuge. The longer the time taken for the boundary to become diffuse during the course of

Table 4. *The degree of normality of samples of hyaluronic acid as judged by various criteria*

The symbols range from + + + and + + to indicate normality to − indicating gross abnormality.

Sample	Clinical state of joint	Quantity of fluid	Normality of mucin clot	Diffuseness of boundary	Protein concentration/$\eta_{rel.}$	$(1/S_{20})/\eta_{rel.}$	A/G ratio	Intrinsic viscosity	$(1/S_{20})$/ mucin content	$\eta_{rel.}$/ mucin content
Ox	+ +	+ +	+ +	+ + +	+ +	+ +		+ + +	+ +	+ +
J	+ +	+ +	+ +	+ +	+ +	+ + +	+ +	+ +	+ +	+ +
A	+ +	+ +	+ +	+ + +	+ +	+ +	+ +	+ + +	+ + +	+ + +
C	+	+	+	+ +	+	+	+	+	+	+
D	+	+	+	−	+	+	+	−	+ +	+
E	+	+	+ +	+	+	+	+	−	+	−
F	+	+	+	+ +	+	+	+	−	+	−
G	+	+	−	+	−	+	+	−	+	−
H	−	−	−	+	−	−	−	+ +	+ + +	+ ±
K–N	−	−	±		−	+	−			

a run at a given hyaluronic acid concentration, the more normal the hyaluronic acid was considered. (5) The quantity of protein present divided by the viscosity. The protein content of a normal fluid is lower than that of a pathological effusion. The protein concentration was compared with the viscosity to correct for dilution of the synovial fluid. (6) $1/S_{20}$ compared with Ostwald viscosity. This was based on the residual variation observed from the ultracentrifugal results of Fig. 2. (7) Albumin/globulin ratio. High values of the A/G ratio were taken as indicating normality. (8) The intrinsic viscosity measured by Couette or Ostwald viscosimeter. High values were again taken as indicating normality of the hyaluronic acid. (9) The value of $1/S_{20}$ compared with mucin content. The more normal fluids were expected to show a reduced S_{20} value for equivalent mucin concentrations. (10) The Ostwald viscosity compared with the mucin content. A high viscosity for a low mucin content should indicate normality. Owing to the variation of anomaly this is not equivalent to (8). The criteria used in the first seven columns of the table show very close agreement in assessing normality. (The mixed nature of sample *D* has already been mentioned as a possible reason for its appearing very abnormal by the ultracentrifuge boundary test.) Agreement is not close, especially in the cases of samples *D* and *H*, in the last three columns; these columns all involve the use of dry weight of mucin clot. It is likely that this property of clot formation is differently affected by abnormality of hyaluronic acid. Comparison of any other property of hyaluronic acid with dry weight of mucin will then give misleading results. The Couette viscosimeter results are interesting in this connexion. According to most of the criteria of normality sample *H* shows greater abnormality than either sample *C* or *D*. The values in Table 2 indicate this only when assessed by the comparison of the viscosity anomaly with viscosity. This measurement alone of the three tests proposed in Table 2 does not involve the weight of mucin clot.

Further results showed that this comparison of viscosity with viscosity anomaly affords a convenient method of estimating the normality of the hyaluronic acid in synovial fluid (Ogston & Stanier, 1953*b*; Johnston, 1955*b*).

In conclusion, human hyaluronic acid resembles fairly closely that of ox fluid. There is, however, a greater individual variation between apparently normal joints. In the fluid from abnormal joints the ultracentrifugal results, viscosity and mucin formation show variation. As the fluid becomes more abnormal, the ultracentrifugal behaviour, the viscosity and the anomalous viscosity are affected in a simple manner. The weight of mucin formed in pathological fluids still capable of clot formation is affected in a complicated way and the weight of mucin formed is difficult to interpret.

SUMMARY

1. The viscosity, ultracentrifugal behaviour, mucin formation, mucin properties and hyaluronic acid molecular dimensions have been studied for a number of human synovial fluids from normal and pathological knee joints.

2. The ultracentrifugal and viscosity behaviour is affected in abnormal fluids which have not lost the property of clot formation.

3. It is suggested that even in fluids which will form a compact mucin clot, the weight of the clot may be abnormal, and that the use of mucin estimations even in these nearly normal fluids is unsatisfactory.

4. Other criteria of normality for human hyaluronic acid are discussed.

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The Viscosity of Normal and Pathological Human Synovial Fluids

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The use of mucin content as a criterion against which to compare such physicochemical properties of human synovial fluids as viscosity and hyaluronic acid sedimentation constant appears unsatisfactory. The more abnormal fluids will not form compact clots, and this power of clot formation is an insensitive and qualitative test. Even in the more normal solutions which do form compact clots the use of the clot weight appears misleading, and investigation of the mucin formed with respect to specific refractive increment, total N, or glucosamine content appears to show no variation in this normal to near-normal range (Johnston, 1955). The comparison of sedimentation constant with viscosity is a very laborious technique. The investigation of three human fluids by means of a Couette viscosimeter, however, had suggested that the reduction in the degree of non-Newtonian viscosity ('anomaly'), compared to the viscosity at a standard velocity gradient, might afford a test of normality of hyaluronic acid. This reduction in anomaly had been further demonstrated for ox fluids rendered artificially abnormal (Ogston & Stanier, 1953*a*). It was accordingly decided to design a viscosimeter capable of providing varied velocity gradient and requiring very small quantities of fluid, and with this to investigate more fully the viscous properties of human fluids. Full viscosity measurements were carried out on a number of fluids from different sources, and in some cases the effects of temperature, dilution and of the action of hyaluronidase were investigated.

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EXPERIMENTAL

Methods

Viscosity was measured by timing the rate of flow of fluid in a horizontal capillary, variation in the flow rate being obtained by applying different pressures.

Apparatus. The apparatus (Fig. 1) consisted of a horizontal length of capillary tubing (length 16 cm., int. diam. 0·3 mm.) connected to two 5 cm. vertical pieces. These were of 1 mm. int. diam. increasing to cups of about 0·5 cm. int. diam. A cross tube (1 mm. int. diam.), with a tap, connected the two limbs of the U-tube so formed. One limb of the U-tube was connected by a 3-way tap to air and to a water or mercury manometer. The U-tube and manometer were mounted on a rigid frame arranged so that the U-tube could be immersed in a thermostatically controlled water bath to the level indicated. Marks 1, 4 and 8 cm. apart were arranged along the fine capillary.

Procedure. The U-tube was first cleaned thoroughly by washing in succession with conc. HNO_3, glass-distilled water, ethanol, 10% (w/v) KOH in ethanol and finally with ethanol. It was dried, and the fluid to be examined was introduced so that it filled the capillary and cross-tube and rose into the expansion in the side pieces (0·3 ml. of fluid required). A small droplet of *o*-chlorotoluene was then added to the fluid. This was done by dipping a piece of

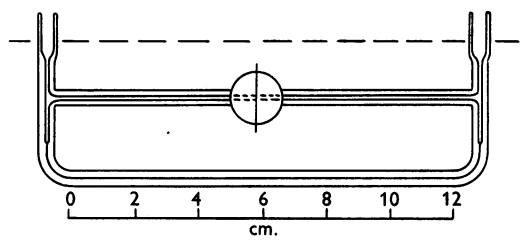

Fig. 1. Section of capillary viscosimeter.