Some Properties of Human and other Synovial Fluids

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The main object of this paper is to report measurements made on a small series of human synovial fluids and on a few sheep and ox fluids. These measurements were made in order to test the two methods suggested by Ogston & Stanier (1953*a*) for detecting differences of the specific properties of the hyaluronic acid as it occurs in synovial fluids. These methods, both of which are based on viscosity measurements, were suggested because (1) the viscosity of synovial fluid depends almost entirely on its hyaluronic acid content; (2) the viscosity and particularly its variation with velocity gradient are sensitive to changes in the state of hyaluronic acid. Because the viscous properties vary rapidly with concentration it is necessary either to compare relationships which are independent of concentration or to measure and make allowance for the concentration. In Ogston & Stanier's first method, the ratios of viscosities at zero velocity gradient and at 50 sec.⁻¹ are plotted against the viscosities at zero velocity gradient; while dilutions of a given sample of hyaluronic acid give points lying reproducibly on a single curve, different hyaluronic acids give different curves. Scott Blair, Williams, Fletcher & Markham (1954) have proposed a similar method. In their second method, the concentration of hyaluronic acid forms the basis for comparing viscosities, which may be measured under any stated conditions of flow.

Sedimentation measurements were also made on a few representative fluids, in order to obtain further information on the state of their hyaluronic acid. It is concluded (i) that there are inter-species and possibly intra-species differences of the hyaluronic acid of synovial fluid; (ii) that these differences are detectable by comparing viscosities in a way not requiring measurement of concentration; (iii) that the measurement of the concentration of hyaluronic acid in human fluids presents difficulties which make comparisons of viscosity on a concentration basis of doubtful value. Having reached these conclusions, we intend, at least for the present, to discontinue this work.

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EXPERIMENTAL

Synovial fluids. Human 'traumatic' fluids were supplied by Mr R. G. Taylor of the Wingfield-Morris Orthopaedic Hospital, Oxford. They were taken at operation from cases of moderate trauma of the knee joint, free from any obvious disease. Fluids containing blood were not used. The volumes of the fluid varied with the state of the joint from ca. 1 ml. to ca. 20 ml. The fluids were bottled with full sterile precautions and were stored at 1°. It was usual for a clot, presumably of fibrin, to form within 24 hr. of collection; this was centrifuged off before use, and the separated supernatant fluid subsequently remained clear. Normally all work on a fluid was completed within 3 days of opening the bottle; no sign of infection or degradation was observed within this period. In a few cases, measurements were repeated after several months' storage, and showed no detectable change of properties. A single sample of fluid taken from a normal joint, about 12 hr. *post mortem*, was obtained. Human pathological fluids were supplied by the Rheumatism Research Unit, Canadian War Memorial Hospital, Taplow. Ox fluids were obtained from the slaughterhouse, as described by Ogston & Stanier (1950); sheep fluids were obtained by the same method, either from the slaughterhouse or from sheep used for experiments at the Nuffield Institute for Medical Research, Oxford; in both cases fluid was taken from the ankle (astragalotibial) joint.

Concentration of hyaluronic acid. A mucin clot was formed by acidification of 0.5–1 ml. of fluid and its dry weight determined, after thorough washing, as described by Ogston & Stanier (1950). The adequacy of the washing procedure was checked. In some cases, where enough material was available, duplicate determinations were made; these agreed within 10%. Dilution of the fluid up to threefold, before precipitation was performed, did not seem to affect the result. Dilutions of the fluids were made by weight with the buffer used by Ogston & Stanier (1950) (0.2 M-NaCl, 0.0077 M-Na₂HPO₄, 0.0023 M-KH₂PO₄).

Sedimentation. This was observed in a Svedberg oil-turbine ultracentrifuge and sedimentation coefficients were calculated by the method of Cecil & Ogston (1948). Measurements were made on successive dilutions by about 1.5-fold, until the hyaluronic acid boundary could be no longer observed.

Viscosity was measured at 25° in the Couette viscometer described by Ogston & Stanier (1953*b*), modified to allow measurements to be made on only 2 ml. of fluid. A smaller pot, internal diameter 1.121 cm. was made to fit into the standard pot, and a smaller bob was used, diameter 1.00 cm. × length 4 cm. The annular gap was 0.0605 cm. The accuracy of this instrument was somewhat less than

with the 10 ml. pot, the error of a single measurement being about 3%. Measurements were made in most instances on the original fluid or (if its volume was insufficient) on a dilution of it. In some cases, measurements were made on a series of dilutions, to allow the intrinsic viscosity to be estimated. Each solution was measured at a number of low velocity gradients, the viscosity at zero velocity gradient being obtained by extrapolating $1/\eta_{\text{rel}}$ against velocity gradient; also the viscosity at velocity gradient 50 sec.^{-1} was estimated by interpolation.

RESULTS

Viscosities. Table 1 gives values of $\log \eta_0$ (η_0 = relative viscosity at zero velocity gradient), $\log \eta_{50}$ (η_{50} = relative viscosity at 50 sec.^{-1}), and $\log \eta_0/\eta_{50}$. The hyaluronic acid content is given as mg. mucin/ml. These values apply to undiluted human fluids, except where dilution was necessary because of the small volume available. Where dilution has been performed, the dilution factor is given. Serial dilutions were made on ox and sheep fluids. In Fig. 1, $\log \eta_0/\eta_{50}$ is plotted against $\log \eta_0$; this is a log version of Ogston & Stanier's (1953a) plot, having the advantage of less extended scales. In Fig. 2, $\log \eta_0$ is plotted against mg. mucin/ml.

Intrinsic viscosities ($[\eta]_c = (\eta_{\text{rel}} - 1)/c$, $c = 0$). These were estimated by extrapolating $\log \{(\eta_{\text{rel}} - 1)/c\}$ against c to zero concentration. In each case, two plots were made, using values of η_{rel} at velocity gradients 0 and 50 sec.^{-1} respectively; these converged to the same intercept at $c = 0$ within 0.1 log units. Values are given in Table 2, c being expressed as g. hyaluronic acid complex/100 ml.; the assumption is made that human mucin is like ox mucin (Ogston & Stanier, 1952) in containing 45% of the complex by weight.

Sedimentation coefficients. The observed values of S were first corrected to water at 20° . Correction was then applied for the effect of protein on the sedimentation of hyaluronic acid by use of the empirical relationship found by Johnston (1955a),

$$(1/S)_{\text{corrected}} = (1/S)_{\text{observed}} - 0.043P,$$

where P is the concentration of protein in g./100 ml., estimated from the sedimentation schlieren diagram. $1/S_{20}$ was then plotted against c (g. hyaluronic acid complex/100 ml.). From these plots values of S_0 (S_{20} at zero concentration) and $d(1/S)/dc$ were obtained, and are given in Table 2.

DISCUSSION

Expression of concentration. All the measurements of concentration have been based on the dry weight of mucin clot. This concentration scale is suitable for the comparison of properties of different synovial fluids and for the extrapolation of S to zero concentration. For the calculation of $[\eta]_c$ and $d(1/S)/dc$, and of quantities derived from them, the

concentration of hyaluronic acid, together with any material with which it is associated in solution, is required. It has been established that ox 'hyaluronic acid' is a complex containing protein, and that this complex forms 45% by weight of mucin. Although similar information is not available for other 'hyaluronic acids', it seems likely that these also occur as a similar complex; this assumption has been made and the term 'hyaluronic acid' is used in this sense.

Non-Newtonian viscosity. The plot of $\log \eta_0/\eta_{50}$ against $\log \eta_0$ (Fig. 1) gives a measure of the relationship of the variation of viscosity with velocity gradient to the viscosity at zero velocity gradient. Ox, sheep and human fluids are clearly different from each other; and there is a suggestion that rheumatoid arthritis fluids differ from other human fluids, both traumatic and pathological.

The deviations, amongst the human fluids, from any common curve are outside the experimental errors and this shows that the properties of the hyaluronic acids in different traumatic fluids vary (cf. Johnston, 1955b). It must be remembered that all these fluids were taken from joints which had suffered varying degrees of trauma; this may have affected the hyaluronic acid, but the series is too small to make any attempt to correlate the degree of trauma and the viscous properties. It is interesting that the single sample obtained *post mortem* gives a point lying well off the mean curve of traumatic fluids.

Viscosity and concentration. Fig. 2 again shows clear differences between ox, sheep and human fluids, but the points for the human fluids are very much more scattered than those in Fig. 1. Little of this scatter can be due to errors of determining either η_0 or the mucin content of a given fluid. It must therefore be ascribed, either to variation from fluid to fluid of the viscosity due to a given concentration of hyaluronic acid (if the mucin content measures this correctly); or to variation from fluid to fluid of the proportion of hyaluronic acid in mucin. Of these possibilities, the second seems the more likely, and might be caused by variations of the qualities or amounts of protein or ionic constituents of the fluids (cf. Sundblad, 1950). Whatever the cause of this variation, it does not seem seriously to affect the relationship between viscosity and its variation with velocity gradient; one must conclude that while this relationship forms a suitable basis for comparing the specific properties of hyaluronic acids in synovial fluids, the comparison of viscosity with mucin content does not.

Sedimentation coefficients and intrinsic viscosities. The values both of $d(1/S)/dc$ and of $[\eta]_c$ for human hyaluronic acids, given in Table 2, are affected by the uncertainty about the estimation of concentration by mucin clot formation, noted above. These

Table 1 (a). *Concentrations and relative viscosities of synovial fluids from human joints*

Fluid	Dilution	Concentration (mg. mucin/ml.)	$\log \eta_0$	$\log \eta_{50}$	$\log \eta_0/\eta_{50}$
Human fluids from traumatized knees					
Tr	1/7.77	1.54	0.73	0.63	0.094
Ta	—	8.3	2.48	1.80	0.68
Mi	1/1.82	5.8	1.55	1.28	0.28
Mi	1/3	3.5	1.10	0.95	0.14
Sa	—	6.4	1.37	1.12	0.25
Sh	—	8.3	2.09	1.51	0.58
Mo	—	5.9	1.18	0.97	0.21
Ba	1/2.06	6.7	2.46	1.68	0.78
Bul	—	5.8	1.74	1.33	0.41
Bur	—	10.0	1.74	1.45	0.30
Wo	—	8.6	2.16	1.60	0.56
Va	1/1.59	5.2	2.05	1.45	0.60
Pev	1/2	6.6	2.12	1.54	0.58
Du	1/2	—	1.39	1.18	0.21
Pathological human fluids					
Br (1)	—	4.9	1.64	1.36	0.28
(2)	—	9.4	2.17	1.61	0.56
(3)	—	6.2	1.31	1.20	0.11
(4)	—	5.5	1.71	1.41	0.30
(5)	—	6.9	2.03	1.58	0.44
(6)	—	5.2	1.44	1.25	0.19
(7)	1/1.28	5.9	1.54	1.31	0.23
Pe	—	5.3	2.13	1.59	0.54
La, Rheumatoid arthritis, untreated	—	—	0.92	0.86	0.06
La, after cortisone	1/2.61	—	0.88	0.80	0.08
Wa, Disseminated lupus erythematosus, treated cortisone	—	—	0.89	0.82	0.07
Coo, Reiter's syndrome, untreated	—	4.3	1.84	1.36	0.48
Cor, Pulmonary osteoarthropathy, untreated	—	6.1	2.11	1.53	0.58
Ja, Osteoarthritis	—	7.0	2.56	1.72	0.84
Post-mortem fluid (normal)	—	4.7	2.48	1.53	0.95

Table 1 (b). *Concentrations and relative viscosities of synovial fluid from joints of sheep and ox*

Fluid	Dilution	Concentration (mg. mucin/ml.)	$\log \eta_0$	$\log \eta_{50}$	$\log \eta_0/\eta_{50}$
Sheep ankle fluids					
A	—	5.8	3.14	1.70	1.44
B (1)	—	5.0	2.33	1.42	0.91
(2)	1/1.66	3.0	1.57	1.09	0.48
C (1)	—	5.4	2.50	1.52	0.98
(2)	1/1.62	3.4	1.73	1.19	0.54
(3)	1/1.95	2.8	1.46	1.06	0.40
(4)	1/3.30	1.65	0.98	0.79	0.19
Ox joint fluids					
Ankle fluid 64 (1)	—	2.04	1.21	0.82	0.39
(2)	1/1.47	1.38	0.86	0.63	0.23
(3)	1/2.01	1.01	0.68	0.51	0.17
Ankle fluid 65 (1)	—	4.93	3.00	1.50	1.50
(2)	1/1.31	3.75	2.29	1.31	0.98
(3)	1/1.80	2.73	1.73	1.10	0.63
Ankle fluid 61	—	2.22	1.54	1.02	0.52
Knee fluid 29 K (1)	1/1.70	2.60	1.70	0.99	0.71
(2)	1/2.56	1.73	1.21	0.80	0.41
(3)	1/5.1	0.87	0.77	0.51	0.26
(4)	1/10.3	0.43	0.44	0.30	0.14
(5)	1/20.5	0.22	0.14	0.09	0.05
Ankle fluid 37 A (1)	—	1.82	1.54	0.98	0.56
(2)	1/1.50	1.21	1.12	0.78	0.34
(3)	1/2.25	0.81	0.82	0.60	0.22

Table 2. Sedimentation coefficients, intrinsic viscosities, and molecular dimensions of hyaluronic acids

Fluid	$10^{13} \times S_0$	$10^{-13} \times d(1/S)/dc$	$[\eta]_c$	$10^{-6} \times M$	V'	J
Human, traumatic						
Bul	8.7	2.0	17	2.6	195	7
Bur	6.1	1.1	13	1.2	61	14
Wo	5.4	1.5	22	1.2	68	18
Va	5.2	1.3	36	1.3	120	30
Pe	9.5	2.3	30	3.7	220	10
Br (4) Rheumatoid arthritis	6.7	2.6	17	1.7	195	7
Sheep ankle fluid (C)	11.5	2.6	35	5.5	320	8
Ox	14.7*	3.3*	55*	10	520	8

* From Ogston & Stanier (1952).

Fig. 1. Ordinate $\log \eta_0/\eta_{s0}$, abscissa $\log \eta_0$ (see text) for various synovial fluids, plotted from Table 1. $\bullet$, Ox; $\bullet$, sheep; $\times$, human traumatic; $\circ$, human rheumatoid arthritis; $+$, other human pathological fluids; $\bullet$, normal human fluid obtained *post mortem*.

values are therefore less reliable than those for ox and sheep hyaluronic acids. The values of S_0 for sheep and human hyaluronic acids may be affected by the high protein content of these fluids (ca. 4 g./100 ml.) which, as Johnston (1955*a*) has shown, causes irregularity of the $1/S-c$ plot; this error is unlikely to exceed 10% of S_0 and cannot account for the whole of the differences between different samples.

There appear again to be marked differences of properties between ox, sheep and human fluids, while the latter show considerable variations amongst themselves.

Fig. 2. Ordinate $\log \eta_0$ (see text); abscissa concentration in mg. mucin/ml. for various synovial fluids, plotted from Table 1. $\bullet$, Ox; $\bullet$, sheep; $\times$, human traumatic; $\circ$, human rheumatoid arthritis; $+$, other human pathological fluids.

It is noticeable that, amongst the group of human fluids, the estimated values of $d(1/S)/dc$ and of $[\eta]_c$ do not run parallel. It is therefore likely that errors of concentration do not contribute the whole variation of either of these quantities, and that there are some genuine differences from sample to sample. This confirms the conclusion drawn from Fig. 1 that different hyaluronic acids differ in their specific properties. Our values of S_0 and $d(1/S)/dc$ for human fluids fall within the same range as those reported by Johnston (1955*b*).

Particle dimensions. Ogston (1953) has shown that indications of particle dimensions can be obtained from the measured values of S_0 , $d(1/S)/dc$ and $[\eta]_c$. Such values must in any case be accepted with caution and this should be increased, in the present case, by the relative uncertainty of the experimentally determined quantities for human and sheep fluids. Nevertheless, a comparison of the particle dimensions of hyaluronic acids from

different sources is probably valid. The striking differences between the values of the (unsolvated) molecular weight M , shown in Table 2, are certainly real. The large values of the hydrodynamic specific volume V' are consistent with the random-coil form of particle, deduced for ox hyaluronic acid by Ogston & Stanier (1951) and, as would be expected, V' varies in parallel with M . Values of the ellipticity J are in some cases rather large for a simple random-coil, but it is doubtful whether any conclusion can be drawn from this. It should be noted that the values of V' and J given in Table 2 for ox hyaluronic acid differ from those of Ogston & Stanier (1952) because of the new choice of constants noted by Ogston (1953).

The reality of the differences of M are shown by the fact that considerable amounts of hyaluronic acid present in a human traumatic fluid passed through a glass filter which entirely prevented the passage of ox hyaluronic acid.

SUMMARY

1. Viscosity measurements have been made on a small series of traumatic and pathological synovial fluids from human knee joints; also on three samples from ankle joints of normal sheep.

2. Sedimentation measurements have been made on a few of these fluids.

3. Comparison of variation of viscosity with velocity gradient, with its value at zero velocity gradient, suggests the existence of inter-species and

possibly intra-species differences in the specific properties of hyaluronic acid. Sedimentation measurements appear to confirm this and suggest that human hyaluronic acids have considerably lower particle weights ($1-4 \times 10^6$) than those of sheep ($5-6 \times 10^6$) or ox (10^7).

4. Specific properties of human synovial fluid hyaluronic acid cannot, at present, be satisfactorily compared on a basis of concentration estimated from weight of mucin.

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Metabolism of β -Mercaptoethylamine

1. IN MICE

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Bacq *et al.* (1953) have shown that 3 mg. of β -mercaptoethylamine injected immediately before irradiation protect mice against 700 r. of X-rays. The percentage of ^{35}S remaining in the body of a mouse at various times after intraperitoneal injection of 3 mg. [^{35}S] β -mercaptoethylamine has previously been determined (Verly, Bacq, Rayet & Urbain, 1954), but for the understanding of the duration of the very short-lived radioprotection, it is more interesting to measure the ^{35}S that is still

present in β -mercaptoethylamine (cysteamine) or β : β' -diaminodiethyldisulphide (cystamine) molecules. This paper presents the results of such measurements.

EXPERIMENTAL

Synthesis of [^{35}S] β -mercaptoethylamine hydrochloride

This compound was prepared as follows (see also Mills & Bogert, 1940; Bestian, 1950). Ba^{35}S was ground in a mortar with some water, then transferred to an H_2S generator where it was made to react with an excess of phosphoric acid.

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