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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.

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. HNO₃, 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.

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finely drawn glass tubing, first into the fluid in the viscosimeter, then into the *o*-chlorotoluene and finally back again into the fluid. By capillarity a drop of *o*-chlorotoluene was obtained in a column of fluid and it could be expelled below the surface of the fluid in the viscosimeter. The droplet could then be brought into the fine capillary by adjusting the flow or by gravity. The apparatus was not very sensitive to the size of the droplet, and one of suitable size could readily be judged. The U-tube was then connected to the manometer and frame and immersed in the bath. Readings were made as follows. The tap of the cross-tube of the viscosimeter was left open and the 3-way tap connected to air, viscosimeter and manometer until the level in the two limbs reached equilibrium. Both taps were then closed and the required pressure was obtained in the manometer. The 3-way tap was turned to connect manometer and viscosimeter, and the droplet in the fine capillary was timed between two suitable marks by stop watch. Both taps were opened for re-equilibration and the procedure was repeated. Alternate positive and negative pressures could be applied during an estimation.

Calibration. Trouble was first experienced through the sticking of droplets in the capillary. *o*-Chlorotoluene was found to be more satisfactory than mercury (Smithies, 1953). Extreme cleanliness of the capillary was required but acid cleaning was adequate so long as the capillary was afterwards exposed to KOH solution in ethanol. Experiments with water showed that, if the droplet completely filled the bore of the capillary but its length was not greater than 3 times its diameter, no difference in the flow was observed with different droplets. Only occasional water runs were carried out to check reproducibility. Fig. 2 shows a plot of applied pressure against reciprocal flow time for water, points obtained on four successive calibration runs being shown. The slope of this line was used to calculate relative viscosities. The upper curve shows points obtained with a synovial fluid.

Calculation of relative viscosity. The relative viscosity at any flow rate was taken as the ratio of the pressure required for the fluid to that required by water. Zero velocity gradient

Fig. 2. Variation of the reciprocal of the flow time in 8 cm. of the capillary with the pressure applied. Curve A: for synovial fluid. Curve B: for water.

viscosities were estimated by extrapolation. A plot of $1/\eta$ against $(100/t)^2$ (where t = flow time in sec. for 8 cm. capillary) was reasonably linear at low flow rates. Infinite velocity gradient viscosities were estimated by extrapolation (plot of η against t), and by measuring the slope of the plot of pressure against $1/t$ where this showed linearity at the higher flow rates. These estimates agreed closely and when possible the mean was used.

Comparison with Couette-type viscosimeter. The viscosimeter was compared with a Couette-type viscosimeter (Ogston & Stanier, 1953b). Fluids whose viscosity had been measured with the Couette viscosimeter at Oxford were kindly provided by Dr A. G. Ogston and Dr J. E. Stanier and viscosity measurements were made on these with the capillary viscosimeter. Values for zero velocity gradient viscosity showed reasonable agreement, but the values obtained with the capillary instrument tended to be higher. Values for infinite velocity gradient were all considerably lower in the capillary instrument. It had been previously noted that Ostwald viscosimeters frequently gave lower viscosities than Couette infinite velocity gradient values and also the capillary instrument can be used up to higher velocity gradient than the Couette viscosimeter. Capillary measurements of infinite velocity gradient viscosities may therefore be preferred. (Ogston & Stanier, personal communication, agree that their extrapolation (1950) to infinite velocity gradient is probably unreliable.)

Difficulty arises in the capillary instrument, however, due to the fact that the velocity gradient in capillary flow is not uniform. It is therefore possible for a fluid with a high anomaly but a low viscosity at infinite velocity gradient to show the same capillary flow time as one with a reduced anomaly but higher value at infinite velocity gradient. An attempt was therefore made to try to assess the errors that this might involve. Values for the viscosity obtained at a standard Couette velocity gradient (44.2 sec.^{-1}) were compared with the flow time in the capillary viscosimeter required to give the same viscosity. Good agreement for flow time was obtained with fluids of differing viscosity and anomaly. This indicated that the mean velocity gradient for the capillary flow time was not affected seriously by variation in the anomaly of the fluid examined.

Viscosities obtained in the case of nine fluids at velocity gradients in the Couette viscosimeter of 14.7 and 73.5 sec.^{-1} were compared with capillary results. It was found that these viscosities agreed very closely with viscosities obtained at flow times of 100 and 20 sec. (s.d. of duplicate estimations, $\pm 3.8\%$). The flow times were therefore, within experimental error, inversely proportional to the Couette velocity gradients, suggesting a satisfactory linear proportion between 'mean velocity gradient' and flow time in the capillary viscosimeter. These results were used to convert the capillary flow time to mean velocity gradient.

Materials

Synovial fluids. Both ox and human synovial fluids were used. They were collected and stored as previously described (Johnston, 1955). Fluids were centrifuged but not dialysed. Dilution was made by weight with standard buffer (0.2M -NaCl; 0.0077M - Na_2HPO_4 ; 0.0023M - KH_2PO_4 ; pH 7.3).

Hyaluronidase solution. Benger's 'Hyalase' freshly made up in buffer was used.

***o*-Chlorotoluene.** This was re-distilled at 40° under reduced pressure.

RESULTS

The viscosities obtained at five different velocity gradients for synovial fluids are shown in Table 1. Post-mortem samples (PM₁₋₄) were obtained from the knee joints of subjects who had not suffered

from any disease likely to produce joint abnormality. Clinical oedema of the legs was absent in all cases. They were collected between 6 and 24 hr. after death. With two exceptions, rheumatoid arthritis samples (RA₁₋₉) were obtained from the knee joints

Table 1. *The viscosities at different velocity gradients and temperatures of normal and pathological human synovial fluids*

Sample	Dilution factor	Temp. (°)	η_0	$\eta_{14.7}$	$\eta_{73.5}$	η_{368}	η_{∞}
PM ₁	0.331	0	34.0	14.5	8.10	4.48	3.38
		10	34.8	14.1	8.35	4.48	3.55
		20	37.5	15.2	9.15	5.50	3.75
		25	25.0	14.1	8.83	5.28	4.10
		30	23.5	13.7	8.70	5.65	4.70
		37	20.5	12.8	8.95	5.80	4.95
		45	21.0	13.6	9.40	6.10	5.25
		25	3.76	3.20	2.80	2.28	2.14
	0.113	45	3.04	2.77	2.45	2.45	1.88
		25	2.38	2.20	2.08	1.90	1.83
	0.079	37	2.35	—	2.04	1.83	1.79
		45	1.90	1.90	1.90	1.90	1.90
	1.000	25	1250	110	48.0	20.8	13.5
		37	790	113	51.2	23.7	15.0
	0.330	25	10.0	8.00	7.50	5.30	4.60
		37	10.1	8.10	6.47	4.90	4.13
PM ₂	0.167	25	3.77	3.20	3.20	2.72	2.55
		37	3.30	2.91	2.84	2.42	2.24
	1.000	25	5000	187	55.0	17.7	7.70
PM ₃	1.000	25	7000	233	77.0	24.0	9.00
Ox	1.000	25	33.3	15.5	9.24	4.91	3.90
RA ₁	1.000	25	48.8	26.8	17.9	10.8	9.00
		37	42.3	25.9	16.2	10.8	8.75
		45	27.7	22.7	18.2	11.6	8.90
	0.500	25	8.07	6.80	5.68	4.65	4.22
		37	6.78	6.08	5.40	4.35	3.60
		45	7.80	6.42	5.83	4.58	3.94
	0.249	25	2.80	2.40	2.68	2.40	2.32
		37	2.03	2.03	2.03	2.03	2.03
	1.000	25	87.0	36.0	21.2	11.9	9.30
		37	44.6	28.5	20.2	11.6	8.75
		45	47.1	29.2	20.4	12.0	8.90
	0.538	25	10.5	8.80	6.63	5.07	4.24
		37	9.20	7.60	6.22	4.75	3.80
	0.270	25	3.30	2.80	3.05	2.72	2.30
		37	2.77	2.77	2.77	2.77	2.77
RA ₂	1.000	25	111	27.2	15.4	9.30	7.50
		37	34.0	22.4	15.2	9.65	7.60
		25	48.5	29.2	20.0	12.0	10.2
	0.538	25	172	67.2	34.5	17.5	12.2
		25	29.4	17.2	14.2	9.75	8.30
	0.270	25	41.7	23.6	16.0	10.6	7.80
		25	200	62.0	34.0	18.3	12.2
	1.000	25	8.70	8.00	7.00	5.30	4.50
		12.5	5630	240	95.5	37.5	19.5
	0.740	25	1920	252	106	41.3	18.1
		37	1320	243	106	42.6	18.4
		25	540	133	59.0	26.5	15.2
	0.538	37	338	122	61.0	28.9	16.0
		25	200	57.6	32.8	16.0	11.6
		37	149	55.0	30.5	16.6	11.4
RA ₃	0.363	25	37.0	25.3	15.4	9.50	7.30
		37	33.4	21.3	15.2	9.50	7.47
		25	9.30	7.20	5.45	4.20	3.85
	0.181	25	125	61.0	34.4	18.0	12.1
		37	105	54.5	34.6	18.8	12.4
	1.000	25	200	58.0	31.2	15.4	9.90
		25	100	34.0	18.9	10.0	6.50
M ₁	1.000	25	5630	240	95.5	37.5	19.5
RA ₄	0.740	25	1920	252	106	41.3	18.1
		37	1320	243	106	42.6	18.4
		25	540	133	59.0	26.5	15.2
	0.538	37	338	122	61.0	28.9	16.0
		25	200	57.6	32.8	16.0	11.6
		37	149	55.0	30.5	16.6	11.4
	0.363	25	37.0	25.3	15.4	9.50	7.30
		37	33.4	21.3	15.2	9.50	7.47
		25	9.30	7.20	5.45	4.20	3.85
	0.181	25	125	61.0	34.4	18.0	12.1
		37	105	54.5	34.6	18.8	12.4
	1.000	25	200	58.0	31.2	15.4	9.90
		25	100	34.0	18.9	10.0	6.50
M ₂	1.000	25	5630	240	95.5	37.5	19.5
M ₃	1.000	25	1920	252	106	41.3	18.1
M ₄	1.000	25	1320	243	106	42.6	18.4

The subscripts attached to the column headings give the values of the velocity gradient in sec.⁻¹.

of subjects who were not in an acute phase of the disease and who were not being treated with cortisone. (RA₃ was obtained during an acute phase and RA₅ from a Baker's cyst near the knee.)

A miscellaneous group of fluids was also used. These consisted of fluids obtained at operation on knee joints for a foreign body (M₁) and for torn cartilage (M₂). Sample M₃ came from a case of pulmonary osteoarthopathy and M₄ from a case of Reiter's syndrome.

From Table 1 it will be seen that the viscosities of the undiluted fluids vary considerably, pathological fluids showing reduced viscosity. It is not clear, however, whether this reduction in viscosity is due to dilution or abnormality of the hyaluronic acid.

Fig. 3 gives an indication of the degree of viscosity anomaly compared to the viscosity at a standard velocity gradient. It will be seen that the ox and post-mortem fluids show an increased anomaly relative to the pathological fluids. The fluids in the miscellaneous group tend to be intermediate in their properties between the 'normals' and the rheumatoid arthritis fluids. Dilution of the fluids shows that the relative increase in anomaly in the case of the normals is not abolished by dilution.

Effect of temperature. In many cases the viscosity of the fluid was measured at more than one temperature. The results are included in Table 1. It will be seen that the viscosity at high velocity gradients is

Fig. 3. Variation in the extent of the non-Newtonian viscosity of synovial fluid (measured as $\eta_{14.7 \text{ sec.}^{-1}}/\eta_{368 \text{ sec.}^{-1}}$) with $\log \eta_{368 \text{ sec.}^{-1}}$. ●, Ox fluid; ○, post-mortem fluids; ×, post-mortem fluid treated with hyaluronidase; ■, miscellaneous group fluids; □, rheumatoid arthritis fluids. —, Connects points obtained by serial dilution of the same fluid; ---, connects points obtained by treatment of a single fluid with hyaluronidase.

unaffected by temperature while at low velocity gradients viscosity is reduced by a rise in temperature. It is only at very low velocity gradients that any temperature difference is apparent. The viscosity at a velocity gradient of 75 sec.^{-1} is unaffected, and systematic changes only appear at a gradient of about 20 sec.^{-1} or less. The fall in viscosity with temperature rise becomes proportionally greater the more viscous the fluid. This is illustrated by Fig. 4 which compares the value of η_0^{25} with the difference in the η_0 values for 25° and 37° . There is no apparent difference in the viscosity behaviour between fluids of the three groups in relation to temperature.

Effect of hyaluronidase. Fluid PM₃ appeared normal when tested by the $(\eta_{14.7} - \eta_{368})/\eta_{368}$ test and

Fig. 4. Variation in the difference between the zero velocity gradient viscosities measured at 25° and 37° with the zero velocity gradient viscosity at 25° . ○, Post-mortem fluids; □, miscellaneous group fluids; △, rheumatoid arthritis fluids.

Fig. 5. Decrease in the viscosity of synovial fluid at different velocity gradient on treatment with hyaluronidase. ○, Viscosity at velocity gradient of 14.7 sec.^{-1} ; ▽, at 73.5 sec.^{-1} ; □, at infinite velocity gradient.

was chosen for a hyaluronidase experiment. 'Hyalase' solution was added to the fluid, bringing the final 'Hyalase' concentration to approximately 33 Benger units/100 ml. The fluid was mixed, transferred to the viscosimeter and measurements carried out at intervals during the next 26 hr. (The solution was kept in the water bath for 7.5 hr., returned to 4°, and replaced in the bath on the following day.)

Fig. 5 shows the fall in viscosity with time. It will be noticed that the viscosity at high velocity gradients was unaffected for some time, although the low velocity gradient viscosities immediately fell rapidly. The infinite velocity gradient viscosity did not begin to decrease until 7.5 hr. after the addition of the hyaluronidase; during this time the zero velocity gradient viscosity fell from 5300 to 13.5. This rapid decrease in the anomaly relative to high velocity gradient viscosity is illustrated in Fig. 3.

DISCUSSION

Apparatus. The simple viscosimeter appears to give satisfactory results in the case of human synovial fluid. It has the advantages of requiring very little of the fluid to be tested and having a considerable mean velocity gradient range. Any errors involved in the use of capillary flow appear to be negligible.

Results. The comparison of the extent of the viscosity anomaly of a fluid with its viscosity at a standard velocity gradient appears to give a satisfactory and sensitive test of the normality of the hyaluronic acid in a human synovial fluid. Some of the normal fluids tested show properties similar to those of ox fluids, but others show a reduced anomaly. This could be due to either of two possibilities. (1) That the fluid found in normal joints does not contain hyaluronic acid of fixed properties, but merely of viscosity properties lying within a range, the upper limit of which approximates to the properties of ox fluid. (2) The methods used to overcome the difficulty in obtaining normal fluids have not been adequate. In the case of the miscellaneous and rheumatoid arthritis groups, reduced anomaly was present in all cases. It appears as though the fluid associated with rheumatoid arthritis does not show uniform viscosity properties. This suggests that the hyaluronic acid in rheumatoid arthritis is not so much a distinct abnormal form of hyaluronic acid as a mixture of altered forms. This variation in the anomaly between different samples of rheumatoid arthritis fluids is less, however, than in the case of the normals. The sensitivity of this test in the case of abnormal fluids is indicated by the fact that the fluids of the miscellaneous group and many fluids from the rheumatoid arthritis group were capable of forming compact

mucin clots. This indicates that hyaluronic acid can be altered to give different viscosity properties without an effect on its ability to form mucin.

The fall of viscosity at low velocity gradient occurring as the first detected change in the properties of hyaluronic acid in abnormal fluids may have considerable importance. It has been suggested that the anomalous viscosity is of considerable value in the lubricating powers of synovial fluid (Ogston & Stanier, 1953*c, d*). Thus a fluid which, judged by mucin content and by viscosity measured in an Ostwald viscosimeter, appears normal may be very abnormal when considered as a joint lubricant. On the other hand, the fluid will appear abnormal as judged by this viscosity test.

The treatment of a normal fluid with hyaluronidase led to a steady decrease with time in the viscosity anomaly. The fluid passed through the range of the miscellaneous and rheumatoid arthritis fluids and finally showed less anomaly compared with its viscosity at a standard velocity gradient than any of the other fluids examined. This observed behaviour and the known effect of hyaluronidase on clot formation parallels the properties of the pathological fluids examined. The uncertainty, however, of the precise action of different preparations of hyaluronidase on human hyaluronic acid makes the interpretation of the results doubtful.

SUMMARY

1. A simple type variable velocity gradient viscosimeter of low fluid capacity (0.3 ml.) is described.
2. Measurements of viscosity at various velocity gradients have been carried out on a number of normal and pathological human synovial fluids. The effect of temperature and dilution has been studied.
3. It has been concluded that the reduction of the viscosity anomaly relative to high velocity gradient viscosity is a sensitive test of abnormality in the fluid.
4. The action of hyaluronidase has been shown to cause normal fluids to resemble pathological fluids when tested in this way.

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