2. The respiration of *T. lewisi* depends on the cytochrome system. It is very sensitive to cyanide. It is also inhibited by carbon monoxide, this inhibition being light-reversible.

3. In the absence of extracellular nutrients, *T. lewisi* takes up oxygen at a rate of 40 μl. oxygen/mg. cellular nitrogen (or about 10^9 cells) in 1 hr. at 37°. In the presence of added glucose, mannose, fructose or glutamine, the q_{O_2} value increases from 40 to 600 or more. Glycerol, asparagine and glutamic acid increase the oxygen uptake to a smaller extent.

4. Glucose is oxidized by the trypanosome with an R.Q. of 0·97. For each molecule of glucose utilized aerobically, 3–4 mol. oxygen are taken up, and 1 mol. equivalent of organic acid is produced; half of this is acetic acid, and the rest mainly succinic and lactic acids.

5. The substances which are not utilized by the trypanosome aerobically include: galactose, sorbose, xylose, sucrose, lactose, maltose, lactate, pyruvate, succinate, citrate, α-ketoglutarate, acetate, formate, glycine, alanine and aspartic acid. Added lactate and succinate are not oxidized by intact cells, but lactic and succinic dehydrogenases could be demonstrated in cell homogenates.

6. Anaerobically, *T. lewisi* ferments glucose, the fermentation being stimulated threefold by 0·02M-pyruvate, and 12-fold by 0·016M-bicarbonate. The maximum value of the fermentation rate observed in the presence of carbon dioxide was $q_{CO_2}^{N_2} = 470$. For each mol. of glucose utilized anaerobically, 0·74 mol. carbon dioxide are fixed, and 2·5 mol. equivalents of organic acid are produced. The bulk of the acid accumulating anaerobically is succinic acid (70 %) and lactic acid (15 %); the rest is pyruvic and acetic acid.

7. The anaerobic fermentation is very sensitive to metal-binding substances, being inhibited by about 75 % by 0·0003M-2:2′-dipyridyl and 8-hydroxy quinoline.

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The Dimensions of the Particle of Hyaluronic Acid Complex in Synovial Fluid

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A hyaluronic acid complex was separated from ox synovial fluid by Ogston & Stanier (1950) by ultrafiltration. This complex contained about 30 % protein, and was considered to be the form in which hyaluronic acid occurs in the native fluid; removal of the protein led to degradation. The authors concluded, from their results on the sedimentation and diffusion of this complex, that the mean particle weight is 1×10^6 and the axial ratio about 60. Their values for the intrinsic viscosity indicated an axial ratio of nearly 600, and they interpreted this discrepancy in terms of interaction of the particles. This interpretation is logically unsound, because all the values used had been extrapolated to zero concentration; under this condition there could be no interaction between particles of finite size in a uniform solution.

In this paper some new measurements of viscosity and of streaming birefringence are described. It is shown that these and the former measurements are all consistent with the hypothesis that the particle weight is about 8×10^6, the axial ratio of the order of 1, that is, the particles are nearly spherical, and that the particles are very highly solvated. These properties are, in turn, consistent with the view that the particle of hyaluronic acid complex is a flexible chain.

EXPERIMENTAL

All the measurements were performed on fresh samples of ox synovial fluid, collected as described by Ogston & Stanier (1950) and dialysed against a standard buffer containing 0·2M-NaCl, 0·0077M-Na_2HPO_4 and 0·0023M-KH_2PO_4; pH 7·3.

Viscosity. The viscosities of various dilutions of synovial fluid were measured in a Couette viscometer, whose temperature was thermostatically controlled. A torsional damping device was also added, which greatly reduced oscillations of the inner cylinder and enabled more accurate measurements to be made. Viscosity was measured over a range of velocity gradient between 0·25 and 50 sec.$^{-1}$, extrapolation to velocity gradients 0 and ∞ being performed as described by Ogston & Stanier (1950). Most measurements were made at 25°, but some were also made at 37° and at 0°.

Streaming birefringence. The inner and outer (rotated) cylinders were made of vulcanite, to reduce reflexions. The diameter of the inner cylinder was 2·0 cm., the annular gap was 0·15 cm. and the length of fluid subjected to shear was 5·4 cm. The cylinders were accurately co-axial and vertical. Illumination was from a Pointolite lamp, giving a beam parallel within 0·5°. The polarizer and analyser were large Nicol prisms. The glass plate which closed the bottom of the outer cylinder and the Perspex annulus at the upper surface of the liquid were free from any detectable birefringence. A mica quarter-wave plate was used for measuring double refraction. The upper Nicol and the cross-wires (of Nylon thread) were mounted on circles divided in degrees: the angles of isocline and of extinction could be read to 0·1°.

RESULTS

Viscosity. The results were most conveniently plotted as lines of $\log \frac{1}{\phi}(\eta_{\text{rel.}} - 1)$ against volume fraction of solute, at each value of the velocity gradient (Fig. 1), where ϕ is the volume fraction of solute, assuming zero solvation. The slope of these lines falls with increasing velocity gradient. The intercept of the line for zero velocity gradient at zero concentration is the intrinsic viscosity. The remarkable feature of this set of curves is that they all make nearly the same intercept at zero concentration.

The effect of temperature on the relative viscosity is small, except at the lowest velocity gradients, where a rise of temperature causes a marked fall of relative viscosity (Fig. 2). This is illustrated also in Table 1, where the change of $\log \frac{1}{\phi}(\eta_{\text{rel.}} - 1)$ between zero and infinite velocity gradient provides a measure of the degree of viscosity anomaly. This decreases with rise of temperature, the change being almost entirely due to change of the value at zero velocity gradient.

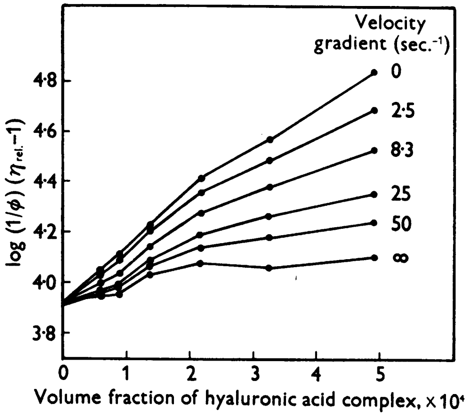

Fig. 1. Variation of $\log \frac{1}{\phi}(\eta_{\text{rel.}} - 1)$ with volume fraction ϕ at different velocity gradients.

The values for the intrinsic viscosity estimated for different samples varied between $10^{3\cdot 8}$ and $10^{4\cdot 0}$, these values being slightly lower than that given by Ogston & Stanier (1950).

Streaming birefringence. Measurements were made on a single (pooled) sample, which was undegraded, as judged by its viscosity. The concentration of the hyaluronic acid complex, including its protein component, was 0·082 g./100 ml. The double refraction was small, and it was not possible to measure the angle of isocline (χ) at a concentration below 0·061 g./100 ml. or the amount of double refraction ($\Delta°$) below 0·041 g./100 ml. These concentrations are high compared with those at which sedimentation and viscosity could be measured. The results are given in Table 2.

DISCUSSION

Evidence bearing on the axial ratio

Viscosity, sedimentation and birefringence. The starting-point of our present interpretation was the discovery that the value of $\log \frac{1}{\phi}(\eta_{\text{rel.}} - 1)$ at zero concentration is almost independent of the velocity gradient (Fig. 1). This can mean only that the particles, at zero concentration, are nearly spherical. It seems that the difference between the extreme

Table 1. $\log \frac{1}{\phi}(\eta_{\mathrm{rel.}} - 1)$ *as a function of velocity gradient and temperature*

(Synovial fluid 36 A, $\phi = 5 \times 10^{-4}$.)

Temp. (°)	Velocity gradient G (sec.$^{-1}$) 0	25	50	∞	Change of $\log \frac{1}{\phi}(\eta_{\mathrm{rel.}} - 1)$ between $G=0$ and ∞
0·5	4·99	4·27	4·15	4·03	0·96
25	4·83	4·33	4·23	4·07	0·76
37	4·63	4·27	4·17	4·03	0·60

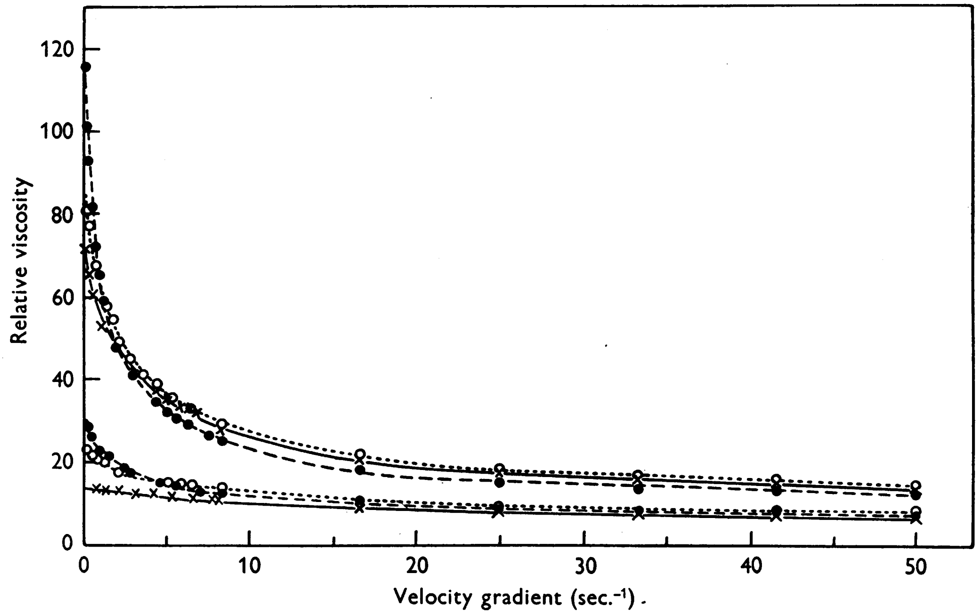

Fig. 2. Variation of relative viscosity with velocity gradient at different temperatures. — — ● — —, 0·5°; - - ○ - -, 25°; — × —, 37°. Upper set of curves, concentration = 0·08 g./100 ml.; lower set, concentration = 0·05 g./100 ml.

Table 2.

(*a*) *Values of* χ *and of* Δ *at* $G = 100$ *sec.*$^{-1}$ *for synovial fluid no.* 44 *at* 25°, *and estimates of the axial ratio* p

Concentration of hyaluronic acid complex (g./100 ml.)	$\eta_{\mathrm{rel.}}$	χ°	Δ°	Estimate of p From χ	From Δ
0·082	8·0	7·5	1·9	7	22
0·061	6·5	8·1	$0{\cdot}8_0$	5	18
0·041	4·2	—	$0{\cdot}3_5$	—	14

(*b*) *Variation of* χ *and* Δ *with velocity gradient* G

Concentration (g./100 ml.)	G (sec.$^{-1}$)	χ°	Δ°
0·082	67	10·2	0·7
0·082	83	9·8	1·2
0·082	100	7·5	1·9
0·082	133	6·1	2·4
0·082	150	6·0	2·9
0·061	67	—	0·5
0·061	83	8·8	—
0·061	100	8·1	0·8
0·061	133	7·9	—
0·061	150	7·5	1·2
0·041	100	—	0·35
0·041	150	—	0·5

values of $\log \frac{1}{\phi}(\eta_{\text{rel.}}-1)\ (\phi=0)$ cannot be greater than 0·1; then, using the theory of Mehl, Oncley & Simha (1940) for zero velocity gradient and that given by Burgers (1938, p. 144) for infinite velocity gradient, it follows that the axial ratio, assuming an elongated ellipsoid, is not greater than 3. The latter theory neglects the effect of Brownian movement, but this is unlikely to affect the conclusion that the axial ratio must be fairly small.

The very high value of the intrinsic viscosity could then be accounted for only by the assumption that the particles are very voluminous, that is, very highly solvated. It will be shown later that this is not inherently unlikely. The values of the effective specific volume V' needed to account for the intrinsic viscosity, using various assumed values of the axial ratio p and the corresponding values of ν given by Mehl *et al.* (1940), are given in Table 3.

Next, using these values of V' and p, the measured value for the sedimentation constant at zero concentration ($13{\cdot}2\times10^{-13}$, Ogston & Stanier, 1950) and Perrin's (1936) values for the frictional ratio f/f_0, values were computed for the molecular weight M and thence for the semi-major axis a (Table 3).

The corresponding expected values of the angle of isocline, were next calculated. The rotational diffusion constant θ_b was first obtained from p and a^3, using Perrin's (1934) equation. A question arose here as to which is the proper value for the viscosity to use in this calculation. Use of the viscosity of solvent gave a range of values of χ which were all larger than the measured values, while use of the viscosity of the solution measured at zero velocity gradient gave too low a range; it was therefore decided to use the measured viscosity of the solution at the concentrations and velocity gradient ($G=100$ sec.$^{-1}$) for which the computations were made. From the values of p and of $\alpha=G/\theta_b$, the values of χ were taken from the tables given by Scheraga, Edsall & Gadd (1949) (Table 3).

To calculate the expected values of the birefringence, Δ° (Foster & Edsall, 1945), the optical constant for the particles (g_1-g_2) was first computed from the equations of Peterlin & Stuart (1939). It was assumed that the particles are optically isotropic, that is, have no intrinsic birefringence; their refractive index was calculated from the measured value of the specific refractive increment of the hyaluronic acid complex (0·00153) and from V'. Δ is then given by the equations of Foster & Edsall (1945):

$$\Delta=\frac{180S}{\lambda}\,\frac{2\pi}{n_l}\,\phi'\,(g_1-g_2)\,f(\alpha,p).$$

S is the optical path length (5·4 cm.); λ the wavelength of light, taken as 546 mμ.; n_l is the refractive index of the solvent, taken as 1·33; ϕ' is the volume

Table 3. *Values calculated on the assumption of various values of the axial ratio*

Axial ratio (p)	Effective specific volume (V' ml./g.)	Molecular weight, M, $\times10^{-6}$	Semi-major axis, a, $\times10^{7}$ (cm.)	$a^3\times10^{15}$ (cm.^{3})	Angle of isocline χ°, $G=100$ sec.$^{-1}$ (g./100 ml.)		Double refraction Δ°, $G=100$ sec.$^{-1}$ (g./100 ml.)			Variation of sedimentation const. $\frac{d\,(1/s)}{dc}$, $\times10^{13}$
					$c=0{\cdot}082$	$c=0{\cdot}061$	$c=0{\cdot}082$	$c=0{\cdot}061$	$c=0{\cdot}041$	
1	$1{\cdot}72\times10^3$	8·8	182	6·0	—	—	—	—	—	15·6
2	$1{\cdot}48\times10^3$	8·7	273	20·3	5·7	6·8	0·017	0·015	0·010	—
3	$1{\cdot}17\times10^3$	8·5	328	35·2	—	—	—	—	—	6·4
5	$7{\cdot}4\times10^2$	8·1	390	58·8	7·1	8·1	0·146	0·108	0·067	2·7
10	$3{\cdot}15\times10^2$	7·2	448	89·5	7·9	8·8	0·44	0·31	0·194	—
20	$1{\cdot}11\times10^2$	6·3	480	110	8·5	9·3	1·34	0·97	0·58	—
50	24·3	5·2	501	125	9·1	10·1	6·1	4·4	2·6	0·04
100	7·2	4·7	512	133	9·3	10·7	19·8	14·0	8·8	—

fraction of the solute, corresponding with the weight concentration and the value of V'; $f(\alpha, p)$ is an orientation factor which has been tabulated by Scheraga *et al.* (1949). Fortunately the predicted values of Δ are rather insensitive to the value used for the viscosity.

Interpolation of the measured values of χ and of Δ in the range of calculated values (Table 3) enables the values of p to be estimated (Table 2*a*). The estimates based on Δ are the more reliable, because they are not seriously subject to the uncertainty about the viscosity, mentioned above. It should be noticed that these estimates are based on measurements at relatively high concentration; their values appear to fall with concentration, so that p might well approach 1 at zero concentration.

Diffusion and osmotic pressure. Ogston & Stanier (1950) found the diffusion constant to be about 1×10^{-7} between concentrations 0·06 and 0·3 g./100 ml. They assumed the same value at zero concentration, supposing that the variations of the entropy of dilution and of the frictional constant would be of approximately equal magnitude and opposite in sign. Experience with polymers of sarcosine (Fessler & Ogston, 1951) led us to investigate this matter more closely. The variation of the entropy of dilution is measured by the ratio

$$\left(\frac{\pi}{v_2}\right)_c \Big/ \left(\frac{\pi}{v_2}\right)_o,$$

where π is the osmotic pressure, v_2 is the volume fraction of unsolvated solute and the subscripts refer to concentration. This ratio was calculated from Huggins's (1942) equation, using a co-ordination number of 6. The calculated value at a concentration 0·184 g./100 ml. is 133. Mr O. Smithies measured the osmotic pressure of a solution of hyaluronic acid complex of this concentration and found 0·66 cm. H_2O. Assuming that the particle weight is 8×10^6, this gives a value for $\left(\frac{\pi}{v_2}\right)_c \Big/ \left(\frac{\pi}{v_2}\right)_o$ of 112, in good agreement with the calculated value.

Since $$\frac{D_c}{D_o} = \left[\left(\frac{\pi}{v_2}\right)_c \Big/ \left(\frac{\pi}{v_2}\right)_o\right] \frac{f_o}{f_c},$$

it should now be possible to calculate the variation of diffusion constant with concentration. However, a difficulty arises over the estimation of the variation of the frictional constant for the case of diffusion. Fessler & Ogston (1951) have given reasons for thinking that the diffusional frictional constant ought to vary with the viscosity of the solution, measured at zero velocity gradient, and that the variation of the sedimentation constant may not be a reliable guide to it. In Fig. 3, the predicted variations of D are shown, calculated using Huggins's theory and both the viscosity and the sedimentation constant to measure f_o/f_c. Although neither curve fits the experimental results very well, the divergence of the upper (viscosity) curve is far beyond the experimental error. Moreover, the value of D at zero concentration, obtained from the lower curve, is $0{\cdot}065 \times 10^{-7}$ and this, combined with the sedimentation constant and $\bar{V}$, gives a value for M of 16×10^6. The agreement of this with the value deduced from viscosity and sedimentation is fairly good, considering the assumptions involved.

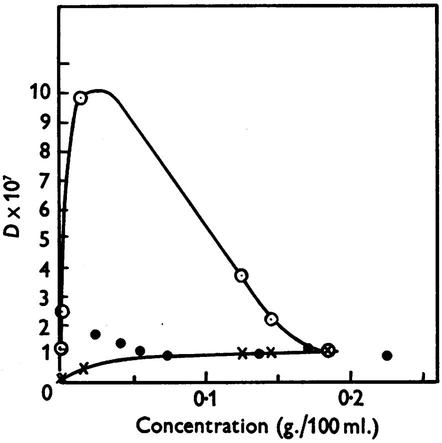

Fig. 3. Variation of the diffusion constant of hyaluronic acid complex with concentration. ●, experimental values of Ogston & Stanier (1950); ⊙, variation calculated from viscosity; ×, variation calculated from sedimentation constant.

The use of the sedimentation constant to measure the variation of the frictional constant for diffusion (rejected by Fessler & Ogston) may perhaps be justified in this case on the grounds of the extremely large size and solvated mass of the particles. This may lead to the physical process of diffusion consisting rather of the movement of solvent into a cloud of immobile particles than of the random movement of particles through solvent; this would resemble the physical process of sedimentation, where the cloud of particles, stationary relative to one another, moves through the solvent.

The variation of the sedimentation constant with concentration. Ogston & Stanier (1950) found that at low concentration $1/s$ varies linearly with the concentration; the slope $d(1/s)/dc$ (c being expressed as g./100 ml.) was $3{\cdot}90 \times 10^{13}$. Fessler & Ogston (1951) have given a theory by which this slope may be related to the values of p, V' and M; values of $d(1/s)/dc$ were calculated for different values of these quantities and are given in Table 3. The observed value is consistent only with a small value of the axial ratio.

General discussion

All the evidence cited, summarized in Table 4, points to the conclusion that the particles of the hyaluronic acid complex present in ox synovial fluid have a low axial ratio. From this it follows that the particles have an effective specific volume of the order of 10^3 ml./g. and an (unsolvated) particle weight between 8 and 9×10^6.

Table 4. *Values of the axial ratio, p*

From	Value
Variation of intrinsic viscosity with velocity gradient	< 3
Angle of isocline	< 5
Birefringence	< 14
Variation of $1/s$ with c	~ 4

The very large effective volume can be understood if the particles are of the nature of flexible chain polymers. Such particles are known to behave as if they were highly solvated because of the entrainment of solvent. The theories of Kuhn & Kuhn (1947) and of Kirkwood & Riseman (1948) predict that a random flexible chain particle would be nearly spherical. With a chain unit length of 6 A. and weight 180 and a particle weight of 9×10^6, Kirkwood & Riseman's theory predicts a mean radius of 140 mμ. which is in fair agreement with our conclusions. Synovial fluid also resembles solutions of chain polymers in showing a marked viscous-elastic behaviour (Ogston, Stanier, Toms & Strawbridge, 1950).

In view of the conclusion that the particles are nearly spherical, and of the slight variation of viscosity with velocity gradient at low concentration, the strong variation of viscosity with velocity gradient at higher concentrations requires explanation. This must presumably be due to interaction of the particles with each other; such interaction is indicated by the viscous-elastic behaviour and by the fact that the large particle volume leads to their being close together even at low values of weight concentration; for example, the volume fraction of particles reaches 1 at a concentration around 0·1 g./100 ml.

Interaction could occur by the entanglement of chains with each other, and this might lead to the linear or network aggregation of particles, both of which would increase the viscosity. Increasing the velocity gradient might have two effects, either of which would reduce the viscosity: (1) the orientation of linear aggregates parallel with the streamlines; (2) if, as is likely, the process of aggregation occupied a finite interval of time, a velocity gradient would reduce the degree of aggregation.

Since the formation of linear aggregates should markedly increase the viscosity, even at high velocity gradient (Burgers, 1938), and since the degree of aggregation must increase with concentration, the relatively small dependence of viscosity on concentration at high velocity gradient (Fig. 1) favours the second mechanism. A rise of temperature may reduce the relative viscosity by causing disaggregation; the much greater effect of temperature on the relative viscosity at low than at high velocity gradient would then also be consistent with the second mechanism, since at high velocity gradient the particles would already be largely disaggregated.

SUMMARY

1. Measurements have been made on ox synovial fluid, dialysed against buffer containing 0·2 M-sodium chloride, 0·0077 M-disodium hydrogen phosphate and 0·0023 M-potassium dihydrogen phosphate (pH 7·3) of: (*a*) the variation of viscosity with concentration, velocity gradient and temperature; (*b*) the variations of the angle of isocline and the birefringence with concentration and velocity gradient at 25°.

2. The results of these measurements are discussed in connexion with data on sedimentation and diffusion. It is concluded that all the evidence is consistent with the particles of hyaluronic acid complex being nearly spherical and very highly solvated; that the hyaluronic acid complex is probably a chain polymer; and that the variation of viscosity with velocity gradient can be explained in terms of interaction between particles.

We are most grateful to Dr J. T. Edsall for a copy of a report (Scheraga *et al.* 1949); to Dr H. Kuhn of the Clarendon Laboratory, for advice and for the loan of optical components; to Dr R. Simha for advice; to Mr O. Smithies for making a measurement of osmotic pressure; and to the Medical Research Council for a Research Grant under which this work was carried out.

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