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The Physicochemical Properties of Two Mucoids from Bovine Cervical Mucin

BY R. A. GIBBONS AND F. A. GLOVER

National Institute for Research in Dairying, University of Reading, Shinfield, Berks

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A study of the phenomenon of flow-birefringence in solutions of macromolecules can yield information relevant to the size, shape and flexibility of the molecules in solution. Essentially this involves observation of the optical properties of a solution under shear, conveniently in a concentric-cylinder viscometer having a transparent base, fitted to a vertical optical bench. Consider a small segment of the annulus with the inner or outer cylinder rotating at constant speed: the flow lines will be along the annulus in a horizontal plane, the direction of the velocity gradient will lie radially across the annulus, also horizontally, and the line of sight will be vertical and at right angles to both. A schematic diagram of a suitable apparatus is given by Glover & Suffolk (1957).

Two factors are involved in the phenomenon of flow-birefringence: the optical and the hydrodynamic.

(1) *Optical factors.* A birefringent body possesses different refractive indices for light polarized in two directions at right angles. Linearly polarized

light passed normally through such a body, in general, emerges elliptically polarized, and cannot be extinguished by rotation of an analyser. If the direction of polarization of incident light is coincident with, or at right angles to, the optic axis of the body, it emerges unchanged and can be extinguished by an analyser. Lines defining the direction of the optic axis are, when applied to a solution under shear, termed neutral lines. The factors which determine the position of the neutral lines in a flowing solution of birefringent molecules will be considered below. Their direction may be located by examining the flowing solution between crossed Nicol prisms. The smaller angle between the neutral lines and the flow lines is called the angle of isocline (χ). The amount of double refraction, that is the difference of refractive indices of the flowing solution for light with electric vectors parallel with and at right angles to the neutral lines, may be measured by means of a quarter-wave plate. It is referred to as the birefringence (Δn) and may be positive or negative.

Flow-birefringence of the type considered here is observed only in solutions of macromolecules which are geometrically anisotropic or which can be deformed in the applied velocity gradient. It will be seen that the effect of the velocity gradient is to cause an alignment, or apparent alignment, of the molecules with their longer axes at an angle between 45° and 0° to the flow lines; this alignment is the origin of the birefringence observed, which is the sum of that due to all the molecules in the field of observation.

The birefringence of a macromolecule arises from two causes: (i) the form or 'Wiener body' birefringence (Wiener, 1912), due only to the geometrical anisotropy of the molecule and to the difference in refractive indices between molecule and solvent, is a function of the square of the difference in refractive indices and is therefore always positive; the optic axis is coincident with the longer geometric axis. For a rigid ellipsoid or rod this is quite straightforward, but for the random-coil molecule the same considerations apply save that in this instance the boundary between the solute particle and solvent is gradual and not abrupt; thus some sort of average difference in refractive indices must be taken (Tsvetkov, 1957). Furthermore, it is necessary that the molecule be deformed into a geometrically asymmetric shape by Brownian motion or by the applied velocity gradient; thus form-birefringence for molecules of this type will be a function of velocity gradient. (ii) The intrinsic birefringence is due to variation in polarizability of the constituent chemical bonds in different directions. There is no presumptive reason why the optic axis of the intrinsic birefringence should coincide with the geometric axis of a rigid body, but it will nevertheless behave as though it does, due to the symmetry of distribution of orientations of the molecules about an axis parallel to the flow lines. In the random-coil molecule, there will be intrinsic birefringence in the deformed condition due to the favoured alignment of parts of the chain in the direction of elongation. This is analogous to strain-birefringence in an isotropic body; the optic axis will be coincident with the geometric axis of distortion. The intrinsic birefringence of a random-coil molecule as well as its form-birefringence will therefore be a function of velocity gradient. The position is not quite so straightforward as this, however, and the question of the position of the optic axis of the intrinsic birefringence of a molecule of this type is considered in detail in the Discussion.

(2) *Hydrodynamic factors.* Rigid molecules in solution when subjected to shear in the type of apparatus indicated will rotate about an axis in the line of sight. If geometrically anisotropic they will

spend a longer portion of their time of revolution in a position in which their longer geometric axes are along the lines of shear than they do in a position with their axes across them (Trevelyan & Mason, 1951). This introduces some degree of order into the sheared solution, in opposition to the effect of Brownian motion which tends to give a random distribution of orientations. As a result there is an apparent (statistical) alignment of the molecules in the solution with their geometric axes at some angle to the flow lines. At low velocity gradients, where the effect of Brownian motion is relatively large, this angle will approach 45° ; as the velocity gradient increases it will approach 0° asymptotically. The behaviour of random-coil molecules, which are not rigid, is more complicated, and it is necessary to consider two limiting conditions, one in which the molecule passes readily from one configuration to another (low internal viscosity), and another in which the energy barriers between one configuration and another are large (high internal viscosity). A molecule of low internal viscosity, as pointed out by Cerf (1951*a*), will behave like an elastic sphere, or liquid drop, and will be distorted at low gradients into an ellipsoid having its major axis at 45° to the flow lines and will become successively more elongated and more closely aligned with the flow lines as the gradient increases (Taylor, 1934; Bartok & Mason, 1958). A molecule with high internal viscosity, on the other hand, having assumed an ellipsoidal configuration, will behave in much the same way as a rigid body. Real molecules probably show behaviour intermediate between these two cases. More detailed and sophisticated treatments of the case of the random-coil molecule are given by Kuhn & Kuhn (1946) and Cerf (1951*a, b*; 1958).

The variation of both χ and Δn with the velocity gradient, G , will in general be of the same form for both the rigid molecule and for a molecule of the deformable, random-coil type; typical curves resemble those in Figs. 3 and 4. In general, rigid molecules give larger birefringences, which increase more rapidly with increasing velocity gradient than do random-coil molecules, but this criterion is inadequate for unequivocal differentiation. Edsall (1942), Lawrence, Needham & Shen (1944) and Cerf & Sheraga (1952) have published reviews on the subject to which the reader is referred for a more complete treatment.

The preceding paper (Gibbons, 1959*a*) describes the isolation of two mucoids from bovine cervical mucin. The flow-birefringence of solutions of these two mucoids has been examined and some anomalous results have been obtained. An explanation of these anomalies is offered and it is inferred that these materials are flexible thread-like molecules of the random-coil configuration. Advantage is taken

of this result to determine the molecular weights and relative expansion factors of these two samples from sedimentation coefficients and intrinsic viscosities. The results give some indication of the factors underlying the difference in physical properties of the native secretions. For a description of this difference see Scott-Blair, Folley, Malpress & Coppen (1941).

EXPERIMENTAL

Materials

Mucoids. These were obtained from two bulked samples of bovine cervical mucus, one obtained from cows in oestrus and the other from pregnant animals. Ultracentrifugally homogeneous mucoid was prepared from each sample as described by Gibbons (1959a).

Solvents. Two solvents were used: (1) aqueous saturated calcium chloride solution-ethanol (9:1, v/v); (2) lithium citrate-citric acid buffer, pH 5.8 at 20°, containing 0.2M-lithium chloride, 8 mM-trilithium citrate, 2 mM-citric acid and 10% (v/v) of ethanol. Solvent (2) was used for ultracentrifugal and viscometric studies and its viscosity was determined in the temperature range 20–30° with calibrated Ostwald viscometers; the density in this temperature range was also determined, by Kohlrausch's method (Short, 1955). Solvent (2) was employed owing to the difficulty of obtaining single-phase solutions in the usual phosphate or acetate buffers.

Methods

Flow-birefringence. The apparatus used was based on that of Ogston & Stanier (1953) and is described by Glover & Suffolk (1957). Observations were made at room temperature and in green light (λ 5461Å) isolated from a mercury arc. The apparatus is designed so that the rate of shear is equivalent to the rate of rotation of the outer cylinder in rev./min., which was measured electronically. It has been observed that mucin may be degraded by subjection to high shear rates, consequently only relatively low rates ($<500 \text{ sec.}^{-1}$), particularly with concentrated solutions, were employed.

Viscosity. The flow-birefringence apparatus may be used as a Couette viscometer. It was modified by installation of a low-speed electric motor in order to enable rates of shear down to 0.2 sec.^{-1} to be applied.

Sedimentation coefficients. A Svedberg oil-turbine ultracentrifuge having the bar-schlieren optical system of Baldwin (1953) was employed. Plates were measured with a travelling microscope and sedimentation coefficients obtained and corrected to water at 20° as described by Svedberg & Pedersen (1940). Concentrations of mucoid from 0.5 to 0.075% were examined.

Partial specific volumes. These were determined pyknometrically in a 10 ml. density bottle, with solutions of

mucoid in buffer at concentrations in the region of 0.25%. The measurements were made in a constant-temperature room at 20°, about 4 hr. being allowed for temperature equilibration. Somewhat lower concentrations than is usual were weighed as it is extremely difficult to avoid inclusions of air if higher concentrations are employed. The accuracy of the figures quoted (Table 1) is probably about $\pm 1\%$.

Results

Flow-birefringence. The variation in angle of isocline and birefringence with velocity gradient is most conveniently shown graphically. Figs. 1–4 show the results for both the oestrus sample and pregnancy sample in solvent (1). Fig. 5 shows both birefringence and angle of isocline curves for the oestrus sample in solvent (2). The curves in Figs. 1 and 2 are anomalous, whereas those in Figs. 3 and 4 are at first sight normal, but there is, at the higher concentrations, a very rapid change in angle of isocline with velocity gradient at the lowest rates of shear examined. The curves in Fig. 5 are again anomalous.

Viscosity. The specific viscosities, $([\eta/\eta_0] - 1)/c$, were plotted against concentration and extrapolated to infinite dilution (Fig. 6); values obtained for specific viscosities at infinite dilution are given in Table 1. At the concentrations (0.12–0.008%) and rates of shear (0.2 – 14 sec.^{-1}) investigated, the mucoid solutions behaved as Newtonian fluids.

Sedimentation coefficients. The reciprocals of the sedimentation coefficients ($1/S_{20,w}$) were plotted against concentration (Fig. 7) and extrapolated to infinite dilution. As may be seen, there is indication of slight curvature in the graph for both mucoids but the best straight line has been preferred for extrapolation purposes. If the curvature is real, the figures quoted for $S_{20,w}^0$ (Table 1) may be slightly low. The schlieren diagrams are shown in Fig. 8.

DISCUSSION

Flow-birefringence

An inversion of sign of the birefringence with increasing velocity gradient (Fig. 2) has already been observed, by Tsvetkov (1955), in solutions of high-molecular-weight polystyrene in dioxan. It has been shown that the form-birefringence of a random-coil macromolecule, such as polystyrene, is a different and more slowly varying function of the velocity gradient than the intrinsic birefringence (Frisman & Tsvetkov, 1958; Copic, 1956, 1957), and it can be seen that if the form-

Table 1. *Physical constants of the two bovine cervical mucoids*

	$10^{18} S_{20,w}^0$ (sec.)	$[\eta]$ (dl./g.)	$\bar{v}$ (ml./g.)	$10^{-6} \times M$
Oestrus sample	20	7.4	0.63	4.16
Pregnancy sample	25	3.2	0.64	3.93

birefringence is, at low velocity gradients, of greater magnitude than the intrinsic birefringence and opposite in sign, the birefringence will, at small gradients, be positive but will become negative as the velocity gradient is increased. Tsvetkov (1957) interprets the curves in this way and adduces some further supporting evidence for this view. No

Fig. 1. Angle of isocline of pregnancy mucoid in aqueous saturated calcium chloride solution-ethanol (9:1, v/v) as a function of rate of shear. Concentrations: 1.2% (○); 0.6% (△); 0.3% (▲); 0.15% (●).

Fig. 2. Birefringence of pregnancy mucoid in aqueous saturated calcium chloride solution-ethanol (9:1, v/v) as a function of rate of shear. Concentrations are as given in Fig. 1.

Fig. 3. Angle of isocline of oestrous mucoid in aqueous saturated calcium chloride solution-ethanol (9:1, v/v) as a function of rate of shear. Concentrations: 0.5% (○); 0.4% (△); 0.1% (▲).

Fig. 4. Birefringence of oestrous mucoid in aqueous saturated calcium chloride solution-ethanol (9:1, v/v) as a function of rate of shear. Concentrations are as given in Fig. 3.

Fig. 5. Oestrous mucoid in ethanolic lithium citrate-citric acid buffer, pH 5.8, concentration 0.3%. Birefringence (△, right-hand ordinate) and angle of isocline (○, left-hand ordinate) as functions of rate of shear.

explanation has been offered for the corresponding angle of isocline curves (Fig. 1), which are again similar in form to those given by Frisman (1958) for high-molecular-weight polystyrene in dioxan. They may be interpreted if the assumption is made that the optic axis of the form-birefringence is coincident with the geometric axis of the deformed molecule, whereas that of the intrinsic birefringence is at a small angle to it. The variation of χ with increasing distortion of the molecule may then be investigated by imagining the two sources of birefringence to be separate and then using the relation of Sadron (1938) to sum them. Thus

$$\tan 2\chi = \frac{F(\beta) \theta_f \sin 2\phi_f - F'(\beta) \theta_i \sin 2\phi_i}{F(\beta) \theta_f \cos 2\phi_f - F'(\beta) \theta_i \cos 2\phi_i},$$

θ_f and θ_i are the form and intrinsic birefringences, and ϕ_f and ϕ_i are the orientations of the optic axes

Fig. 6. Plot of intrinsic viscosity against concentration at rates of shear between 0.2 and 14.0 sec.⁻¹. ○, Oestrous mucoid; ●, pregnancy mucoid.

Fig. 7. Plot of $1/S_{20,w}$ against concentration. ○, Oestrous mucoid; ●, pregnancy mucoid.

of the form and intrinsic anisotropies to the flow lines. β is a function of G , and for a given polymer is a constant multiple of it. F is a function for which different values are given by Copic (1957) and Frisman & Tsvetkov (1958), but in either case it varies more slowly with increasing β than F' , which is $\beta(1+\beta)^{1/2}$. Consider the case where $\phi_f < \phi_i$; then $\sin \phi_f < \sin \phi_i$, but $\cos \phi_f > \cos \phi_i$.

When β is low, F and F' have similar values so that as θ_f is numerically greater than θ_i both numerator and denominator will be positive and $\tan 2\chi$ will be positive. As β is increased, $F'(\beta)$ increases more rapidly than $F(\beta)$, so that at some point the numerator becomes zero; but because of the inequalities set out above, the denominator is still positive. At this point $\tan 2\chi = 0$, $\chi = 0$. Further increase of β results in $\tan 2\chi$ becoming negative, since the numerator will be negative whereas the denominator is still positive. The point at which the denominator is zero is then approached; here the numerator is negative and finite and $\tan 2\chi = \pm\infty$, so that χ now descends to -45° . Further increase in β gives both numerator and denominator negative, hence χ is positive and decreases from 45° asymptotically to zero.

Under the conditions given then, the curves of χ against G would have the form found experimentally by Frisman (1958) and in this investigation. The effect on the angle of isocline as ϕ_i approaches ϕ_f , that is, becomes closer to the geometric axis of

Fig. 8. Tracings of the ultracentrifuge schlieren diagrams for (a) oestrous mucoid, 0.3%, and (b) pregnancy mucoid, 0.5%; in ethanolic lithium citrate-citric acid buffer, pH 5.8, at 270 000 g and 25°. Exposures 15 min. after reaching full speed. The menisci are indicated by arrows.

the deformed molecule, is to reduce the range of values of velocity gradient over which $\tan 2\chi$ descends from a positive value through zero to $-\infty$ and from $+\infty$ to the same positive value; that is $d\chi/dG$ becomes very large in this region. When ϕ_i coincides with ϕ , this part of the curve theoretically becomes vertical, but at this point the birefringence will now be zero, so that this part of the curve will be unobservable. Quite a small inclination of ϕ_i to ϕ , will explain the observed peculiarities so that their origin may be sought in a disturbance which is quantitatively small.

Experimentally it is found that the birefringence is zero at velocity gradients in the region where χ is $\pm 45^\circ$. From the equation given by Sadron (1938) it can be seen that this implies that the sine terms are small compared with the cosine terms. At this point therefore both ϕ_r and ϕ_i are small and the axis of the deformed molecule is close to the flow lines.

A possible source of the discordance between the optic axes of the intrinsic anisotropy and the geometric axis lies in the peripheral portions of the molecule. In both translation and shear the greater part of a random-coil type of molecule may be regarded as immobilizing the solvent within it. The semi-empirical relations of Kuhn & Kuhn (1952) indicate that the radius of the equivalent sphere in shear depends primarily upon the number of statistical chain elements in the molecule and also (but to a much smaller degree) on the ratio of the length to thickness of the chain element. In most cases of interest (that is, where the number of statistical chain elements is large), the equivalent sphere will comprise over 95 % of the molecule. The velocity gradient within the greater part of the molecule will therefore be sensibly zero, whereas in the peripheral parts it will increase radially. The chain elements in the peripheral regions will therefore tend to be orientated in this velocity gradient. The orientation of statistical chain elements due to a velocity gradient within a random-coil molecule has not received much consideration in the past since the effect is likely to be quantitatively small in most cases of interest. Cerf (1954) has considered the problem with the simplifying assumption that the material within the domain of the molecule behaves as a homogeneous liquid, the dynamic birefringence being assumed to be at 45° to the internal flow lines. Rigorous treatment of the problem of the differential orientation of chain segments involves complicated hydrodynamic and statistical considerations, but the following reasoning will give a qualitative indication of the result of this effect. The molecule may be approximated to an impermeable elastic sphere, in the immediate vicinity of which there are a number of very much smaller free-draining chain molecules. The sense in

which these small molecules will be orientated is shown diagrammatically in Fig. 9, both for the sphere (to which the macromolecule may be approximated at vanishingly small gradients) and for an ellipsoid of revolution (into which the macromolecule will be deformed at higher gradients). In both cases the macromolecule will tend to rotate as shown, giving rise to local velocity gradients in the periphery, which will cause orientation of the small molecules as indicated. With the sphere, considerations of symmetry indicate that the local velocity gradient at the periphery of the sphere, due to its rotation, will be proportional to $\cos 2\theta$ (Fig. 9), and therefore the resultant orientation of the small molecules will be at 45° to the flow lines. With the ellipsoid of revolution, which is here shown with its major axis aligned with the flow lines, the resultant orientation of the small molecules is not obvious by inspection but it can be seen that it will in general be at non-zero angle to the major axis of the ellipsoid, and the result is the same if the major axis of the ellipsoid is not aligned with the flow lines.

The important conclusion is that the parts of the chain in the peripheral regions will be orientated in a different manner from those parts in the main bulk of the macromolecule, where there will be a preferred direction of orientation along the major axis of the ellipsoid. A component of the birefringence will be thus introduced which is not coincident with this axis. If the parts of the molecular chain in the peripheral regions are

Fig. 9. Diagram to show the orientation of the peripheral portions of a random-coil molecule in a flow gradient.

assumed to behave as separate free-draining coil molecules, the amount of birefringence from this source will increase as the square of the local velocity gradient (Kuhn & Kuhn, 1945), and may reasonably be considered as part of the intrinsic anisotropy of the molecule. The result of the contribution of the peripheral parts of the molecule (which will be quantitatively small compared with the overall intrinsic birefringence), will be that the optic axis of intrinsic birefringence will be rotated by a small angle to the geometric axis in a direction away from the flow lines. This is a result of the absence of any sharply defined boundary between the domain of a random-coil molecule in solution and the solvent.

Thus far a single molecule has been considered, implying infinitely dilute solutions. It can be seen that with increasing concentration the point on the G axis where $\chi = 0$ moves towards lower values of G . Frisman & Tsvetkov (1958), who also observed this, suggest that as concentration increases the average difference in refractive index between a molecule and the solvent decreases owing to the increasing amount of overlap between the domains occupied by adjacent molecules. As a result, the contribution due to the form-birefringence does not increase linearly with concentration once the concentration has reached the point where there is contact between adjacent molecules. The intrinsic birefringence, on the other hand, is unaffected by this factor. Other points should be considered, however; for example, increasing concentration will result in higher local velocity gradients, due to molecular rotations, for a given macroscopic value of G , which will result in correspondingly greater molecular distortion; also, at higher concentrations, the permeability of the molecules must increase.

The birefringence curves for the oestrous mucoid in solvent (1) do not show the anomalies of the pregnancy sample, nevertheless the angle of isocline does, in the more concentrated solutions, increase very sharply at low gradients (Fig. 3), showing some resemblance to the curves for pregnancy mucoid at somewhat higher gradients (Fig. 1). It is possible therefore that the inversion of sign of birefringence and angle of isocline may be occurring at rates of shear lower than those measured, and where the birefringences are too small for detection. This is a possibility which should be considered when extrapolating the angle of isocline to zero rates of shear (Cerf & Sheraga, 1952). The birefringence is in this case negative, so that it is predominantly due to intrinsic birefringence; it may therefore be concluded that the form-birefringence is smaller than with the pregnancy mucoid. This could be due to a difference in molecular weight but the sedimentation and

viscosity data (see below) do not suggest this. It appears from equation (3) in Tsvetkov (1957) that the form-birefringence will vary inversely as the 'volume' of the domain of the macromolecule, that is, inversely as the cube of the molecular-expansion factor (Flory, 1953). A similar relation is implicit in Copic's (1957) calculations. It will be seen that the sedimentation and viscosity data confirm that the oestrous mucoid is a more expanded molecule than the pregnancy mucoid. Gibbons (1959*b*) has suggested that this difference is chiefly responsible for the differences in physical properties of the native secretions.

The curves in Fig. 5 for the oestrous mucoid in solvent (2) are again anomalous. Similar curves were found by Gray & Alexander (1949) for aluminium laurate in benzene, but these authors found measurable birefringences at zero shear rates, which were not unequivocally observed in this case. Solvent (2) is a much poorer solvent than solvent (1) so that form-birefringence may be appreciable in this case; it should be noted that the birefringences are now positive. If the same sort of reasoning as has been used to elucidate the form of the birefringence curves in Figs. 1 and 2 is applied to where the form and intrinsic birefringences are of the same sign, curves similar to those in Fig. 5 can be obtained. A change of sign of the intrinsic birefringence implies extensive and intimate association of solvent with solute in one of the solvents; in view of the composition of solvent (1) and the known affinity of Ca^{2+} ions for sugars this is not unlikely. Kwart & Shashoua (1958) give other evidence for the interaction between mucin and alkaline-earth ions. The curves may also be accounted for if large aggregates are present. Although there is no indication of such aggregates from ultracentrifugal analysis, Joly (1958) has found evidence that they may arise as a result of the applied shearing stresses. The interpretation of these curves may be left open for the present.

It should be noted that the evidence presented can be most reasonably interpreted in terms of the flexible-coil molecule as a model. Polystyrene, which behaves similarly, is known to be of this form. This suggests that the mucoids examined here are also of this form, though a moderate degree of chain-branching would probably not affect their properties appreciably. This is an important conclusion since it becomes possible to make use of the rather extensive knowledge now available of the physicochemical and thermodynamic properties of this type of molecule (Flory, 1953).

Molecular weights

We have determined the molecular weights by means of the relations of Kuhn & Kuhn (1950), as

modified by Kuhn, Moning & Kuhn (1953), assuming that the number of statistical chain elements is large. This is justified in view of the large molecular weights and relatively low intrinsic viscosities.

With symbols defined as follows (Kuhn *et al.* 1953): λ_t , translational resistance factor; N_m , number of statistical chain elements in the molecule; A_m , length of a statistical chain element; L , extended length of molecule; N_A , Avogadro's number; S_0 , sedimentation coefficient at infinite dilution; η_0 , viscosity of solvent; ρ , density of solvent; $\bar{v}$, partial specific volume; $[\eta]$, intrinsic viscosity; ϕ , volume fraction of solute; r , effective radius of molecule:

$$S_0 = \frac{M(1 - \bar{v}\rho)}{\eta_0 L N_A \lambda_t}.$$

Where N_m is large, $1/\lambda_t = 0.136\sqrt{N_m}$. Putting $L = A_m N_m$ this becomes

$$S_0 = \frac{M(1 - \bar{v}\rho) 0.136 \sqrt{N_m}}{N_A \eta_0 A_m N_m} = \frac{M(1 - \bar{v}\rho) 0.136}{N_A \eta_0 A_m \sqrt{N_m}}.$$

Considering now the intrinsic viscosities,

$$[\eta] = 2.5 \phi \text{ (Einstein, 1911)} = 0.025 \frac{4}{3} \pi r^3 \frac{N_A}{M}.$$

Taking r to be $0.46 A_m \sqrt{N_m}$ (Kuhn, 1948), we have

$$A_m \sqrt{N_m} = \frac{1}{0.46} \left(\frac{30[\eta] M}{\pi N_A} \right)^{\frac{1}{3}},$$

where $[\eta]$ is expressed in decilitres/g. Therefore

$$S_0 = \frac{M^{\frac{1}{2}}(1 - \bar{v}\rho) 0.0625}{N_A^{\frac{1}{2}} \eta_0 \left(\frac{30[\eta]}{\pi} \right)^{\frac{1}{3}}}.$$

This relation is numerically almost identical with the Flory (1953) relationship, if the constant $\phi^{\frac{1}{3}} P^{-1}$ is taken to be 2.5×10^6 . If Stokes' law is assumed in translation, and molecular weights are calculated from equation 14 in Ogston (1953) putting $J = 1$, somewhat higher values for M are obtained. The relevant data are set out in Table 1. It is important that the molecular weights reported refer most probably to the lithium salt (Kent, Record & Wallis, 1957), assuming the acidic groups in the mucoids to be sialic acid residues and that the pK_A of combined sialic acid is of the same order as that of the free acid (Svennerholm, 1957).

Expansion factor

It can be seen that the molecular weights of the two samples are not significantly different, but their intrinsic viscosities differ by a factor of about 2. This implies that under the conditions of experiment the oestrous mucoid is about 25% more

expanded than the pregnancy mucoid (Flory, 1953). This figure may not have much quantitative significance when applied to the mucoids as secreted, but the oestrus secretion is a visco-elastic gel containing about 0.5% of non-diffusible solid, whereas the pregnancy mucin is a thick almost rubbery plasto-elastic material, the concentration of non-diffusible solids being a good deal higher (3–4%). A higher expansion factor for oestrus material is therefore indicated (Gibbons, 1959b), although both materials in the native state are, in aqueous solvents, below their Θ point (Flory, 1953) and form two phases when mixed with water. The pregnancy secretion usually swells somewhat in water, suggesting that the presence of salts in the secretion affects the expansion factor. The presence of extraneous material, such as proteins, may also affect it, for, though there is no very obvious change on adding bovine serum albumin or globulin to mucin, protamine produces a rather dense gel with both mucoids.

The factors affecting the physical properties of mucin are considered in more detail by Gibbons (1959b); and the chemical properties of these two mucoid specimens are described in the preceding paper (Gibbons, 1959a).

SUMMARY

1. Sedimentation, viscosity and flow-birefringence measurements have been carried out on two samples of bovine cervical mucoid.

2. Anomalous flow-birefringence curves have been found which have been interpreted as indicating molecules of the random-coil form.

3. Sedimentation and viscosity measurements have been used to estimate molecular weights, assuming a random coil having a large number of statistical chain elements. Molecular weights of the order of 4×10^6 have been found for both materials.

4. The specific viscosity of the sample isolated from animals in oestrus is about twice that of the sample isolated from pregnant cows, indicating that the oestrous mucoid is more expanded than the pregnancy mucoid.

5. The bearing of these data on the physical properties of the native secretion is briefly discussed.

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Fractionation of a Leucocidin from *Staphylococcus aureus*

By A. M. WOODIN*

Sir William Dunn School of Pathology, University of Oxford

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Staphylococcus produces at least three leucocidins, of which that originally described by Pantón & Valentine (1932) is of particular interest since it attacks only white blood cells and not, like the other staphylococcal leucocidins, red blood cells as well. It is also specific to humans and rabbits. Some of the biological properties of this substance have been described by Gladstone & van Heyningen (1957). In the present paper the production of culture filtrates with high leucocidin activity is described and it is shown that this activity depends upon at least two proteins. These have been highly purified.

MATERIALS AND METHODS

Culture filtrates

Organism. The V8 strain of *Staphylococcus aureus* (Gladstone & van Heyningen, 1957) was used. A stock preparation on a tryptic meat-agar slope was made every

month from freeze-dried organisms and cultured overnight on further tryptic meat-agar slopes to provide inocula for the production of culture filtrates.

Comparison of media for production of leucocidin. This was done in rocking T-tubes (van Heyningen & Gladstone, 1953) at 37° with 20 hr. growth. The cultures were clarified by centrifuging and assayed for leucocidin. Growth was estimated by suspending the organisms in 0.85% NaCl soln. and measuring their turbidity.

Large-scale production of leucocidin. This was done by Mr R. Elsworth and Mr R. Telling at the Microbiological Research Establishment, Porton, Wilts. The medium was that described by Gladstone & van Heyningen (1957), with the substitution of Oxoid Casamino acids and a diffusate of Oxoid yeast extract for the corresponding Difco products. Medium (80 l.) was inoculated with 20 l. of seed (prepared in turn from a 1 l. shake flask inoculated from a tryptic meat-agar slope) and the culture grown in a stainless-steel vessel with bottom aeration and stirring. When the maximum leucocidin titre was reached (8–10 hr.) the culture was cooled to below 10°, clarified by centrifuging and the supernatant sterilized by filtration. Solid (NH₄)₂SO₄ was then stirred in to give a 3.5M-solution and the precipitated material collected by filtration through Whatman no. 1 paper and Hyflo-Supercel. The Hyflo-Supercel and

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