

The Viscometric Behavior of Solubilized Calf Skin Collagen at Low Rates of Shear*

(Received for publication, November 8, 1965)

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SUMMARY

Special Ostwald viscometers with unusually long capillaries were used to study the viscometric behavior of calf skin collagen solubilized in citrate buffer at pH 3.44. Graphs of shearing stress with respect to rate of shear show that at very low rates of shear this system displays Newtonian behavior in capillary flow. Under this condition extrapolation of reduced viscosity to zero concentration gives a value of intrinsic viscosity of 27 dl per g, which is higher than previously accepted values determined under non-Newtonian conditions.

The energy of activation of flow of this system is positive and increases with concentration to a maximum value of 5.8 kcal. Entropy of flow is negative and decreases with concentration.

Many viscometric studies of solubilized collagen have been carried out, but with the exception of a single paper by Fessler (1), none of them considered flow at very low rates of shear. The results of these investigations are given in Table I. All investigators used capillary flow viscometers with the exception of Fessler, who worked with a Couette viscometer (1).

The solubilized collagen molecule is a rigid rod 13.8 Å in diameter and 3000 Å long (2). Because of this enormous axial ratio, the solubilized collagen molecule shows a strong tendency toward alignment with a flowing stream, and only at very low rates of shear will Brownian motion supervene and set up a pattern of random orientation. The purpose of this study is to extend the investigation of the viscometric behavior of solubilized collagen preparations to very low rates of shear where this condition prevails; to consider rate of shear dependence, temperature dependence, and concentration dependence; and to determine the energy of activation of flow and entropy of flow.

EXPERIMENTAL PROCEDURE

Skins of 1- to 2-year-old calves served as the natural source of collagen for this research. These were obtained at slaughter, transported to the laboratory packed in ice, and immediately

prepared for solubilization of the collagen. After removal of the hair with an electric clipper and the cutting away of superficial fat, the skins were sliced in a Randall¹ microtome to separate the corium, which was subsequently diced with scissors into approximately 1-inch squares. The skins were kept under ice water except during the actual operations of clipping and cutting.

Solubilization was carried out by keeping the diced skin in contact with approximately its own volume of citrate buffer in a cold room at a temperature of 6–8° with occasional stirring. The buffer had a pH of 3.44 and an ionic strength of 0.64. Methylolate (0.01%) was added as a preservative. As solubilization progressed, buffer ions were removed from the solution by adsorption on the hide and by the Donnan effect. To compensate for this, at regular intervals a portion of the preparation was withdrawn, its pH was measured, and it was titrated against 0.1 N sodium hydroxide. The resulting data indicated the amounts of citric acid and sodium citrate to be added to restore the initial electrolytic state. After 2 weeks, the solubilized collagen preparation was drawn off and centrifuged for 4 hours at 20,000 rpm in the No. 21 rotor of a Spinco model L centrifuge to remove suspended shreds of skin.

The collagen preparations were assayed by direct weighing. The collagen was reconstituted by dialyzing a definite volume to exhaustion of the electrolyte, washing the precipitated material with five changes of acetone, and collecting it on a coarse sintered glass filter. After drying overnight in a vacuum oven at 80°, the reconstituted collagen was weighed.

Verification of the preparation was based on electron microscopy, sedimentation pattern, and differential thermal analysis, which were applied to all preparations used in this study. Electron microscopy was carried out on a small portion of collagen reconstituted by dialysis to exhaustion of the electrolyte. In each case, the electron micrographs showed characteristic collagen fibers almost exclusively (11). A Spinco model E ultracentrifuge equipped with Philpot-Svensson optics was used for sedimentation, and each sedimentation pattern showed a single hypersharp peak with no trailing material in evidence. Differential thermal analysis was performed on a du Pont model 900 thermal analyzer. Each thermogram showed a single transition at 37°, which corresponds to the gelatinization temperature of collagen solubilized in a citrate buffer (2). A sedimentation constant was determined for a limited number of the collagen

* A preliminary presentation of this paper was made at the Ninth Annual Meeting of the Biophysical Society, San Francisco, February 24, 1965.

¹ Mention of commercial items is done for concreteness and does not constitute an endorsement by the United States Department of Agriculture.

TABLE I
Viscosity data on solubilized collagen

Investigators	Natural source of collagen	Solubilizing agent	Rate of shear <i>sec</i> ⁻¹	Temperature	$[\eta]$
Fessler (1)	Rat skin, rabbit skin	Neutral salt solutions	2-50		28-57
Boedtker and Doty (2)	Carp swim bladder	Citrate buffer, pH 3.7	70-200	15-20°	11.5 ± 1.5
Burge and Hynes (3)	Rat skin, perch swim bladder, cod swim bladder	Citrate buffer, pH 1.2 and 3.7	60-210	4-16	13.2-13.7
Gallop (4)	Carp swim bladder	Citrate buffer, pH 3.7		21.4	13.2
		0.5 M MgCl ₂		20.0	16
		0.5 M CaCl ₂		20.0	16
		0.25 M Na ₂ S ₂ O ₃		20.0	16
Noda (5)	Rat tail tendon	Acetate buffer, pH 4.0	ca. 100	20	15
Young and Lorimer (6)	Cod swim bladder	Citrate buffer, pH 3.4		1	13.2
					17.2
Rice <i>et al.</i> (7)	Embryonic calf skin	0.5 % acetic acid followed by freeze-drying, then redissolved in citrate buffer, pH 3.7, and in phosphate buffer, pH 7.4		15	11.5 ± 1.2
Von Hippel <i>et al.</i> (8)	Carp swim bladder	Acid citrate buffer, lyophilized, redissolved in 0.5 M CaCl ₂ , pH 7.0	150	15.65	17.0 ± 2.5
McEwen and Pratt (9)	Rat skin	Citrate buffer, pH 2.99	1100	15.65	15.3
	Rat tail tendon	0.01 M HCl			13.5
		0.1 M HCl			14.5
		0.05 M HCl			16.5
Lewis and Piez (10)	Shark skin	0.5 M sodium citrate, pH 3.5			18.3
					14.2

preparations in concentrations ranging from 0.06 to 0.48%, and this value was 2.8 to 3.0 S referred to water at 20°, which is in agreement with published values (2). As a final guard against progressive deterioration of the collagen preparations used in this study, each day one viscosity determination on each preparation used that day duplicated a run made on the same preparation on a previous day to make certain that the flow time remained the same.

In addition to conventional Ostwald viscometers in sizes ranging from Series 50 to Series 500, specially constructed viscometers having long capillaries bent to a helical shape, as shown in Fig. 1, were used. The capillaries of these viscometers ranged from 1 to 3 mm in diameter and from 78 to 219 cm in length. All viscometers were operated in a thermostated water bath.

The pressure head on each viscometer was set by means of a manostat consisting of two aspirator bottles at different levels connected by a length of Tygon tubing. Pressure was adjusted by varying the vertical distance between the liquid levels in the bottles. This distance was measured with a cathetometer and was used to calculate the total pressure head on the viscometer load. Very low pressure heads were achieved by connecting the manostat to the large diameter limb of the viscometer so that it opposed flow.

To obtain the data required to calculate the shearing stress and rate of shear, an extensive viscometer calibration was necessary. The bulb volume of each viscometer was determined by attaching a graduated pipette by means of a 1-hole rubber stopper to its large diameter limb. The viscometer was filled with water, which was then forced into the graduated pipette by pressure from a rubber squeeze bulb. A reading of the volume of water in the pipette was taken when the liquid level in the viscometer reached each fiducial mark to give the volume of the viscometer bulb.

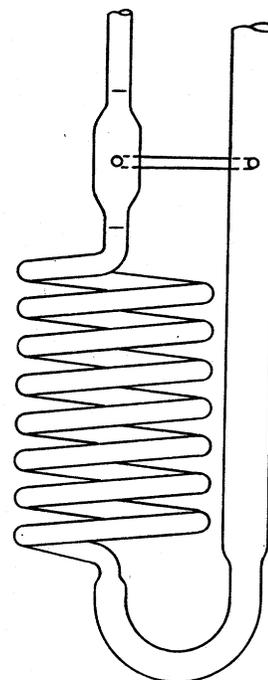


FIG. 1. Typical capillary viscometer with very long capillary to give low rates of shear.

The radius of each viscometer capillary was determined by introducing a weighed slug of mercury. Its length in the capillary was measured with an optical micrometer, and its radius was readily calculated.

The capillary length of those Ostwald viscometers, which had a sharp transition between capillary and adjacent portions of the

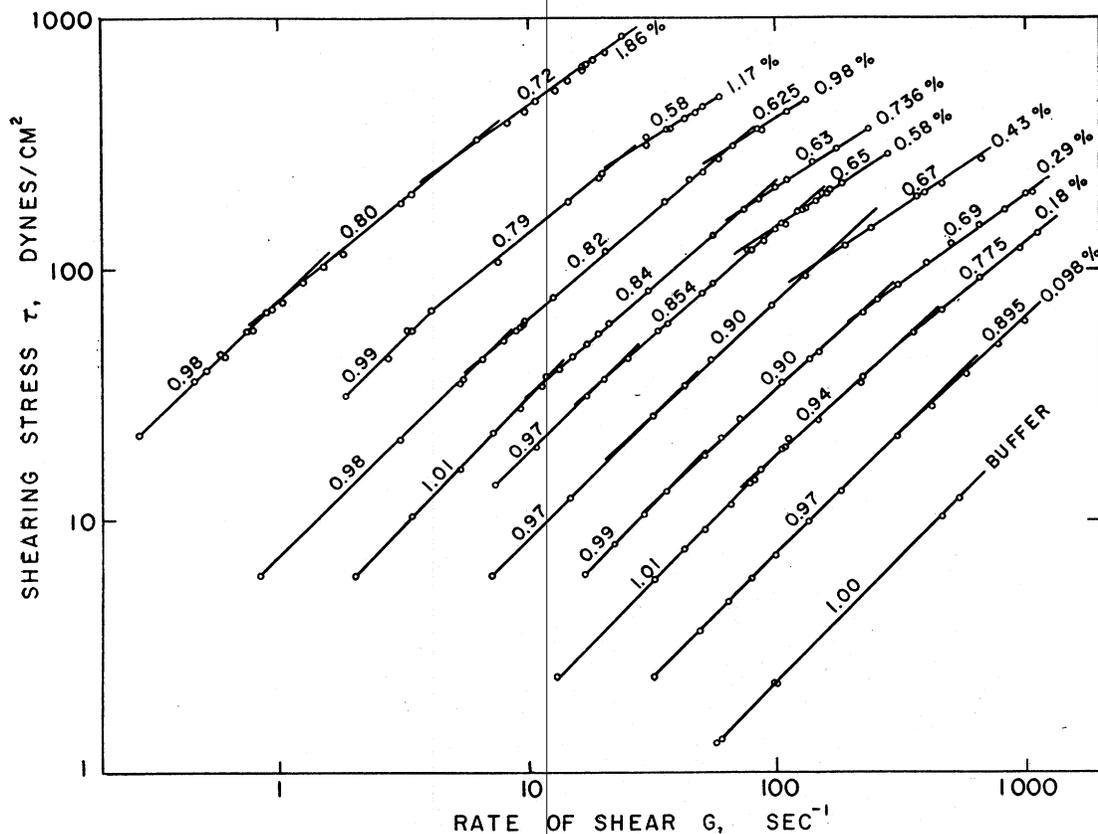


FIG. 2. Plot of shearing stress with respect to rate of shear at capillary wall at 0° for calf skin collagen solubilized in citrate buffer at pH 3.44.

viscometer, was determined by direct measurement with a centimeter scale. In the case of those Ostwald viscometers in which the capillary merges gradually so that its limits are not sharply defined, and in the case of the viscometers with long helical capillaries, length of capillary was measured by allowing a Newtonian liquid to flow through it and then applying Poiseuille's law.

The mean height of each viscometer was determined by an actual run with a Newtonian liquid. The liquid level in each limb of the viscometer was measured with a cathetometer at regular time intervals, and the mean height was obtained from a graph of difference in height with respect to time. The mean height has a slight dependence on total pressure head, and error in its value has its greatest influence at low pressures. Also, error due to surface tension becomes significant at low pressures. This becomes apparent from the fact that the total hydrostatic head is the sum of the heads due to the manostat, the viscometer load, and the surface tension. The last two values become smaller portions of the total head as runs at higher pressures are carried out. To minimize these errors, a new value of mean height was determined for each run at very low total head by measuring liquid levels as the run progressed.

To investigate the necessity of kinetic energy and end effect corrections, Poiseuille's equation incorporating these corrections was applied. This is given by (12)

$$\eta = \alpha Pt - \beta/t$$

where η is viscosity, P is pressure, t is time of flow, and α and β are constants. When Pt^2 is plotted with respect to t , the inter-

cept of the curve on the Pt^2 axis is a measure of the kinetic energy error, and deviation from linearity is a measure of the end effect error. Newtonian liquids with viscosities covering the range observed in this investigation were allowed to flow through several viscometers of each size used, and the above data were plotted. In each case the graph was a straight line that passed through the origin.

RESULTS AND DISCUSSION

Viscosity can be defined by

$$\tau = \eta(G) \quad (1)$$

where τ is the shearing stress, η is the viscosity, and G is the rate of shear. For a Newtonian system η is constant and $f(G) = G$. Shearing stress in flow at the wall of a capillary is given by definition as

$$\tau = \frac{F}{A} = \frac{PR}{2L} \quad (2)$$

where F is the force acting on the mass of liquid, A is the cross-sectional area of the capillary, P is the pressure head, R is the radius of the capillary, and L is the length of the capillary. For a Newtonian system, it can be shown that the rate of shear at the capillary wall is expressed by

$$G = \frac{4Q}{\pi R^3} \quad (3)$$

where Q is the rate of flow. Means of correcting this value for

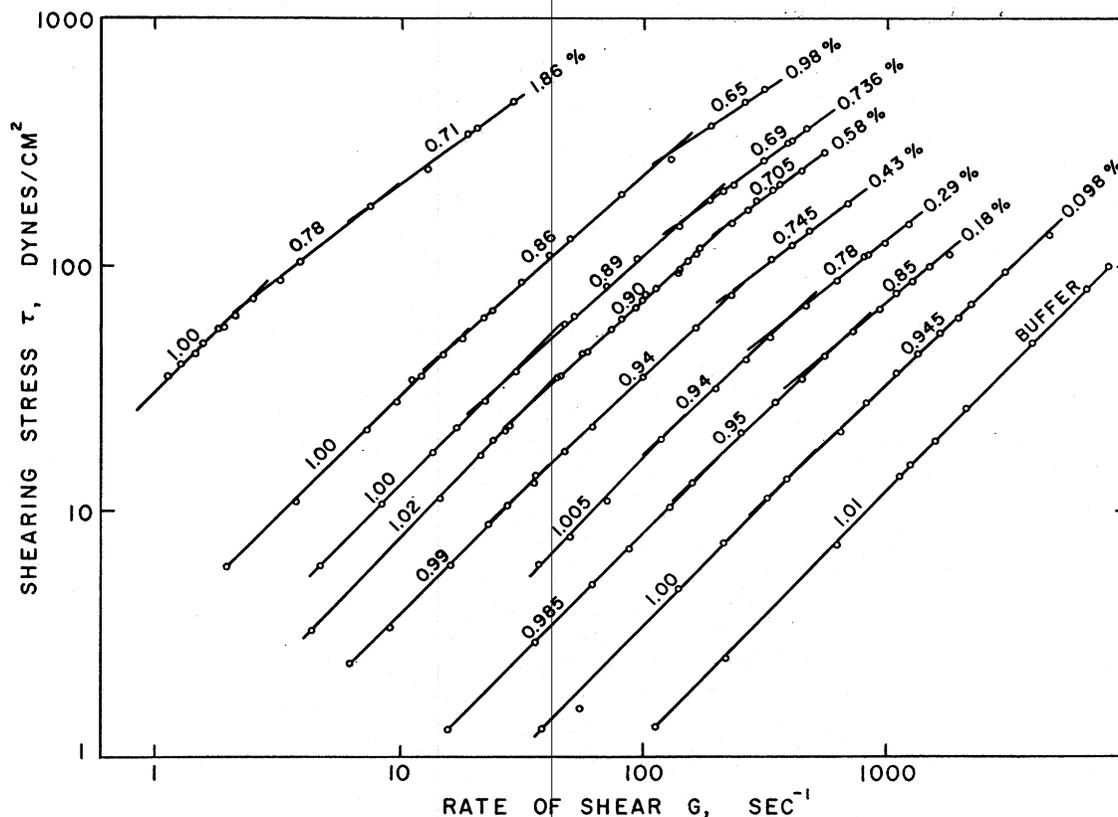


FIG. 3. Plot of shearing stress with respect to rate of shear at capillary wall at 25° for calf skin collagen solubilized in citrate buffer at pH 3.44.

non-Newtonian fluids so that it corresponds to a Newtonian fluid of the same viscosity (at the capillary wall) have been proposed (13, 14).

Shearing stress and uncorrected rate of shear at the capillary wall for calf skin collagen solubilized in citrate buffer are plotted to logarithmic scales for a series of concentrations at 0° in Fig. 2 and at 25° in Fig. 3.

For each concentration of collagen, the plotted points seem to be represented best by a line that is divided by sharp breaks into three straight segments. The slope of each line segment on the logarithmic plot is indicated above it. The concentration of collagen corresponding to each *broken line* is given to the right of it. Although it is difficult to accept the concept of a discontinuous characteristic in viscous flow, these line segments may at least be regarded as tangents to a continuous curve and divide it into three regions of different viscometric behavior. What is especially important to the subsequent discussion is that each line segment at lowest rates of shear be truly straight and have a slope of unity. That this is so was verified by plotting the corresponding points to linear scales, and in each case a straight line passing through the origin resulted.

Linear logarithmic graphs indicate that Equation 1 in this case is equivalent to

$$\tau = \eta G^n$$

where n is the slope of each line segment.²

When n is equal to unity, this becomes the equation for

² Reiner (15) presents an interesting discussion on this type of equation as applied to viscometric data.

Newtonian behavior, as is the case (within experimental error) for each line segment at lowest rates of shear in Figs. 2 and 3. This value of n was, of course, determined under conditions at the capillary wall. However, since Equation 2 is valid for all capillary flow systems, and Equation 3 is obtained by inserting Equation 2 in Poiseuille's law, which assumes Newtonian flow and a parabolic velocity profile across the capillary, Newtonian behavior must prevail at all points within the capillary. The two line segments beyond the first one have successively smaller slopes and thus display pseudoplastic behavior (16), at least at the capillary wall. With the exception of the line representing the highest concentration of collagen, the slopes of the pseudoplastic regions increase progressively with decreasing concentration of collagen and ultimately merge to form a single line segment with a slope of unity; *i.e.* they approach the Newtonian behavior of the aqueous buffer.

The deviation from this behavior observed at the highest concentration employed (1.86%) could be due to particle-particle interaction, or it may be due to not carrying the observations to a sufficiently high value of rate of shear. As concentration of collagen decreases, the Newtonian region extends to higher rates of shear. At 25°, the shearing stress corresponding to any combination of rate of shear and concentration of collagen has a lower value than at 0°.

The data plotted in the pseudoplastic regions are subject to many corrections, which have not been applied because the emphasis in the present paper is on achieving Newtonian behavior by working at very low values of rate of shear. These include the Weissenberg correction (13, 14); correction for end effects (since in this paper it has been ruled out only for New-

tonian systems); and correction for capillary geometry, which may be of such magnitude that it masks the true nature of the flow.

Graphs of reduced viscosity with respect to concentration of collagen are shown in Fig. 4 for data taken at 0° and 25° in the

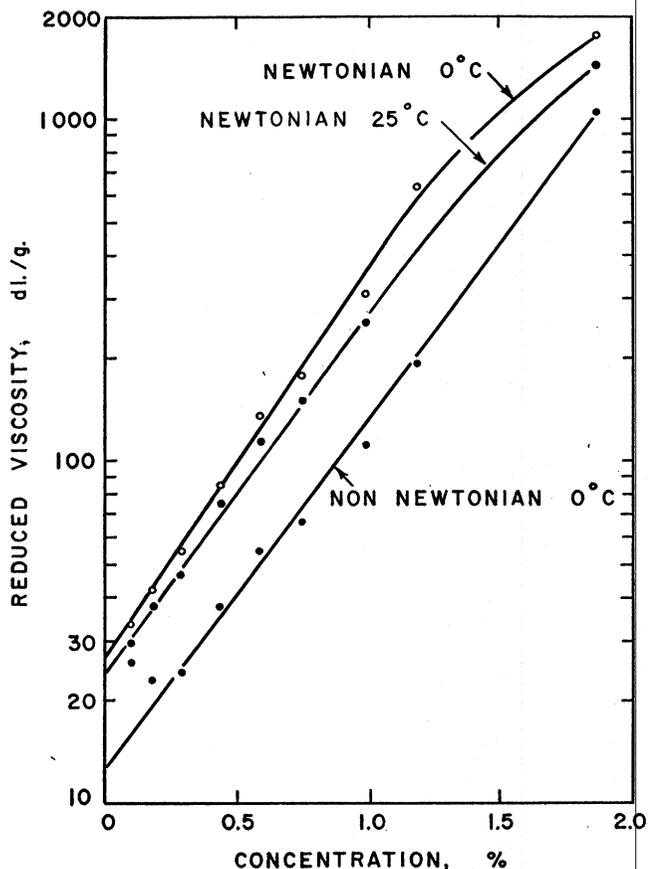


FIG. 4. Plot of reduced viscosity with respect to concentration of calf skin collagen solubilized in citrate buffer at pH 3.44.

Newtonian region and at 0° in the higher of the two pseudoplastic regions. Extrapolation of the 0° Newtonian data to zero concentration yields an intrinsic viscosity for solubilized collagen of 27 dl per g. The 25° Newtonian data extrapolate to a slightly lower value of intrinsic viscosity, although there is sufficient spread of the plotted points to suggest that the data at these two temperatures may actually extrapolate to the same value. When the reduced viscosity values obtained with the data from the higher of the two pseudoplastic regions at 0° are plotted with respect to concentration of collagen, extrapolation to zero concentration gives a value of intrinsic viscosity of about 12 dl per g, which is in agreement with most of the previously published values as shown in Table I.

When the value of intrinsic viscosity of 27 dl per g determined under Newtonian conditions is applied to Simha's equation (17), an axial ratio of 232 results. An intrinsic viscosity of 11.5 dl per g, the previously recognized value, gives an axial ratio of 178. Light scattering data give an axial ratio of 228 (2).

From these considerations it becomes apparent that Newtonian conditions can be achieved in this type of system by reducing the rate of shear sufficiently, and that under Newtonian conditions the true value of intrinsic viscosity will be reached upon extrapolation of reduced viscosity to zero concentration. If viscometric behavior is measured at a rate of shear above the Newtonian region, a lowered value of intrinsic viscosity will result. This should also apply to systems of other suspended particles which are in the form of rigid rods, such as tobacco mosaic virus.

In the non-Newtonian regions there is a partial alignment of the suspended collagen molecules with the flowing stream which gives the flow its pseudoplastic character. Since the degree of alignment is a function of stream velocity and of rate of shear, a relationship between these parameters should lead to a correction that will make it possible to determine intrinsic viscosity under non-Newtonian conditions. This should correct the flow of aligned particles to correspond to the flow of randomly oriented particles. One approach to this problem is via the theory of rate processes (18), which considers flow as a succession of steps

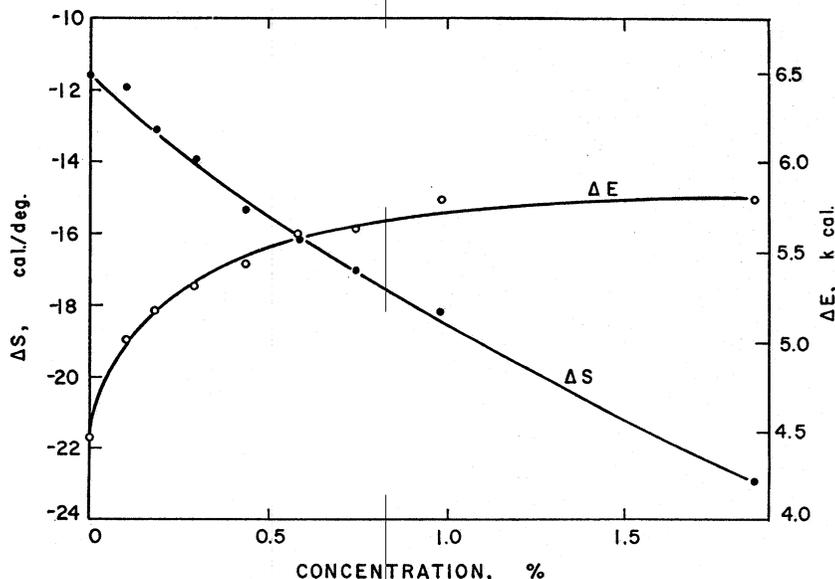


FIG. 5. Graphs of energy of activation of flow and entropy of flow with respect to concentration over the temperature range 0.0-25.0° for calf skin collagen solubilized in citrate buffer at pH 3.44.

of discrete particles advancing into "holes." Because the hole that will accept a rodlike particle moving lengthwise will be quite different from the hole that accepts the same particle when it is moving sidewise or at an angle to the path of flow, the energy of activation of flow and the entropy of flow will be measures of particle alignment.

As a start in this direction, the energy of activation of flow and the entropy of flow for solubilized collagen were calculated over the temperature range of 0–25° with the use of data from the Newtonian region by means of the relation

$$\eta = \frac{Nh}{V} e^{-\Delta S/R} e^{\Delta E/RT}$$

where N is Avogadro's number, h is Planck's constant, V is the molar volume of collagen, ΔS is the entropy of flow, R is the gas constant, ΔE is the energy of activation of flow, and T is the absolute temperature. These values are plotted in Fig. 5. Energy of activation of flow increases with concentration and approaches a limiting value of 5.8 kcal. Entropy of flow is negative and becomes increasingly so with increase in concentration.

Future work that has been planned includes further study of the pseudoplastic regions of the viscosity curves and consideration of velocity profile and effect of capillary geometry, plus a study, analogous to the one presented here, in which a Zimm-Crothers viscometer (19) will be used.

Acknowledgments—The authors wish to thank Robert J. Carroll, Mrs. Anne S. Jahn, Teresa M. Belisario, Mrs. Tatiana Zell, and Charles Marino for their technical assistance.

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