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RHEOLOGY OF FIBRIN CLOTS. IV. DARCY CONSTANTS AND FIBER THICKNESS

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Measurements of small oscillatory deformations of a fibrin clot by axial motion of a rod in a closed tube reveal an anomalous mechanical loss due to permeation of fluid through the clot structure. The Darcy constant for permeation can be calculated from data at the frequency where the apparent storage and loss shear moduli are equal, without the necessity of measurements at much lower frequencies as previously employed. From the Darcy constant, the average number of fibrin monomer units (ν) per cross-section of a fibrous element of the clot can be calculated; it ranges from 4 to several hundred. In the range of fibrin concentration (c) from 3 to 14 g/ℓ, ν is approximately proportional to c^{-2} for clots of coarse structure and to $c^{-0.5}$ for clots of fine structure.

1. Introduction

In the first paper of this series [1], it was shown that small oscillatory deformations of a fibrin clot in annular pumping geometry (a rod moving axially in a cylindrical cell with one end closed) revealed an anomalous mechanical loss associated with permeation of fluid through the clot structure. The magnitude of the permeability, as measured by the Darcy constant, was checked by steady state flow through a clot formed in a cylindrical tube. These measurements enabled us to estimate the average diameter of the fibrous elements of the clot structure in the native hydrated state without the disadvantages of electron micrograph measurements on structures which have been collapsed and dried.

Subsequently, Carr et al. [2] applied the steady-flow permeation method to calculate fiber thicknesses and found that the results at very low fibrin concentrations were consistent with calculations from light scattering. They also showed that the average number of fibrin monomer units per fiber cross-section (ν) could be calculated independently of any assumption concerning the degree of hydration of the protein.

Oscillatory deformations in closed-end pumping geometry have advantages over steady-flow permeation measurements in that the displacements are very small and the pressures are usually very small; the clot is less likely to be altered by collapse or slippage. Originally, the Darcy constant was calculated from the ratio G_a''/ω at low frequencies, where it is nearly independent of frequency and inversely proportional to the Darcy constant. Here ω is the radian frequency and G_a'' the apparent loss shear modulus. Only for coarse clots with relatively high permeation rates could this limiting low-frequency ratio be reached within the experimental frequency range. However, there is an alternative method of calculation which utilizes the value of G_a''/ω at the point on the frequency scale where the apparent storage and loss moduli G_a' and G_a'' are equal; for this method the loss tangent must be small, but that is usually the case. Even for many fine clots with relatively low permeation rates, this point is accessible and so a much wider range of clots can be studied. In the present paper, fiber thicknesses are calculated from additional earlier data and the effects of several variables are noted.

In referring to the character of a clot as coarse or fine, it is recognized that there is a continuous gradation of optical, mechanical, and rheological properties between these two extreme types of structure, but it is convenient to specify these extreme types which are qualitatively different in many aspects of behavior.

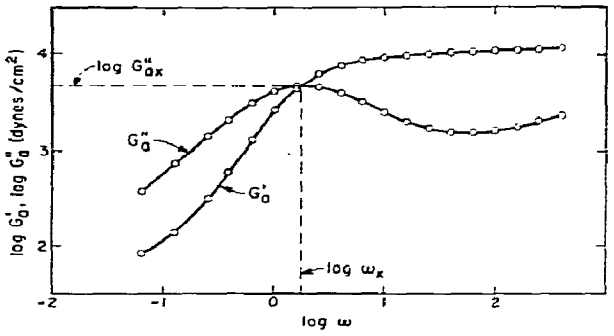

Fig. 1. Logarithmic plots of G'_a and G''_a against radian frequency measured in annular pumping geometry, for coarse clot (pH 7.5, μ 0.16, fibrin 9.3 g/ℓ, thrombin 3.5 u/ml, calcium 0.004 M, with fibrin stabilizing factor).

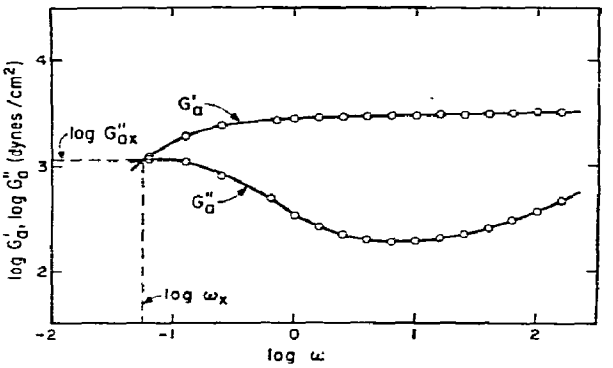

Fig. 2. Logarithmic plots of G'_a and G''_a against radian frequency measured in annular pumping geometry, for fine clot (pH 7.5, μ 0.15, fibrin 5.8 g/ℓ, thrombin 6 u/ml).

2. Theory

A typical plot of the frequency dependence of the apparent storage and loss shear moduli, G'_a and G''_a, observed in annular pumping geometry for a coarse clot is shown in fig. 1. It was previously shown [1] by comparison with observations in open-end annular geometry (where the true viscoelastic moduli G' and G'' are measured) that the sharp drop in G'_a and G''_a at low frequencies does not reflect viscoelastic properties; it is primarily due to permeation of fluid through the clot structure as motion of the inner rod forces fluid to flow through the annulus. The mechanical impedance, or ratio of force to velocity, associated with permeation is taken as

$$Z_p = p\pi R_1^2/v_1, \tag{1}$$

where p is the pressure at the base of the rod, R_1 the rod radius, and v_1 the rod velocity. The total mechanical response can be represented by a model in which Z_p acts in series with an impedance $(A - A_0)(G' + iG'')/i\omega$ and the combination is in parallel with an impedance $A_0(G' + iG'')/i\omega$. (These relations were incorrectly stated in ref. [1] but the equations were correct.) Here A and A_0 are apparatus constants depending on cell and rod dimensions (24,400 and 123 cm respectively in our case). The total observed impedance may also be expressed as $A(G'_a + iG''_a)/i\omega$. The resulting rather complicated equations for frequency dependence of G'_a and G''_a reproduce the behavior observed in fig. 1.

At low frequencies, the equation for G''_a reduces to

$$G''_a = (A_0/A)\,G'' + \omega Z_p/A \tag{2}$$

and under our conditions the first term on the right is comparatively small, so as a good approximation

$$Z_p = A(G''/\omega)_{\text{low }\omega} \tag{3}$$

although this would not hold as $\omega \to 0$ far below the experimental range.

The permeability of a reticulate structure to fluid is expressed by the Darcy constant $\mathcal{D}$, defined [3] as

$$\mathcal{D} = Q\eta_s L/Ftp, \tag{4}$$

where Q is the volume of fluid passing through a column of length L and cross-section area F in time t under a pressure p, and η_s is the viscosity of the fluid. Introduction of our geometry with eq. (1) gives [1]

$$\mathcal{D} = \pi\eta_s L R_1^4/(R_2^2 - R_1^2)\,Z_p, \tag{5}$$

where R_2 is the radius of the cell. Darcy constants were previously calculated by this method, taking Z_p from eq. (3).

A typical plot for a fine clot is shown in fig. 2. Here the permeability is so much smaller that the frequency region where G''_a is directly proportional to ω is experimentally inaccessible. However, Z_p and hence $\mathcal{D}$ is still obtainable by noting the point at which the curves for G'_a and G''_a cross: here, $G'_{ax} = G''_{ax}$ and the frequency is denoted ω_x. If G' and G'' (the true viscoelastic moduli) are known, Z_p can be obtained from a rather complicated expression [4]. The calculation is

greatly simplified if $G'' \ll G'$, which is usually the case; for both coarse and fine clots in this frequency range, $G''/G' < 0.1$. The simple result is then

$$Z_p = CG''_{ax}/\omega, \tag{6}$$

where for our apparatus dimensions $C = 4.83 \times 10^4$ cm if $G'' = 0$ and 5.36×10^4 if $G''/G' = 0.1$. In practice, a value of $C = 5.1 \times 10^4$ cm has been used. A comparison of calculations from eqs. (3) and (6) will follow.

3. Materials and methods

The sources and treatment of human fibrinogen and bovine thrombin were the same as described in the first paper of this series [1]. The measurements of G'_a and G''_a over the frequency range from 0.01 to 160 Hz were made by the Birnboim transducer apparatus as modified by Massa and Schrag [5], as previously described [1]. The pH and ionic strength (μ) were usually 7.5 and 0.15 respectively. (Note that in ref. [1], legend of fig. 4, the ionic strength for the fine clot should read 0.15 and the thrombin concentration for the coarse clot 0.8 u/ml.)

4. Results and discussion

Measurements on a number of clots whose character ranged from moderately coarse to very coarse provided both G''_a/ω at low frequencies and G''_{ax}/ω_x at the crossover point. The Darcy constant (units poise cm^4 $dyne^{-1}$ sec^{-1}) was calculated from both sources with eqs. (3) and (5) or (5) and (6) respectively. From $\mathcal{D}$, the average diameter d of the fibrous elements of the gel structure can be estimated (if the volume fraction ϕ_2 of the gel structure is less than 3%) by the relation [1,3]

$$d = (\mathcal{D}\phi_2/k)^{1/2}, \tag{7}$$

where k can be assigned a value of 0.105 ± 0.01. The corresponding values of d are compared in fig. 3 and show very good agreement between the two methods of calculation. One may thus use the calculation based on G''_{ax}/ω_x [eq. (6)] for clots such as that of fig. 2 where the data from the other method are inaccessible. The numerical data to be cited are all obtained from eq. (6).

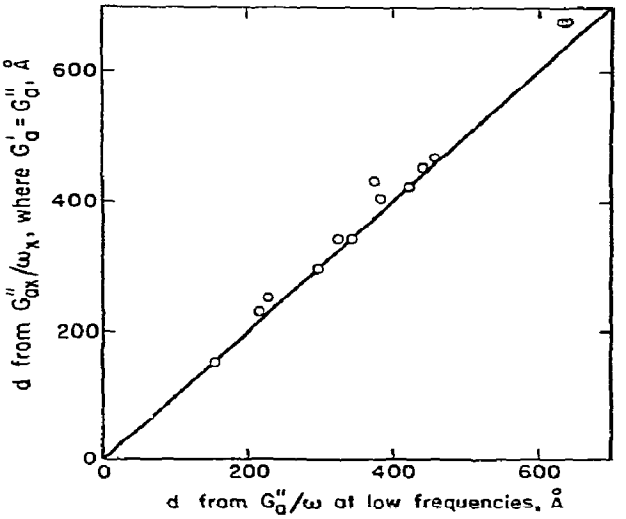

Fig. 3. Comparison of fiber diameters calculated from G''_a/ω at low frequencies [eqs. (3), (5), and (7)] and from G''_{ax}/ω_x at point where $G''_a = G'_a$ [eqs. (5), (6), and (7)].

Although eq. (7) provides the average fiber diameter, there is some arbitrariness in specifying the protein volume fraction ϕ_2. If, as in the calculations for fig. 3, the latter is based on the weight of dry protein ($\phi_2 = 1000\, c/\rho_2$, where c is concentration of dry protein in g/ℓ and ρ_2 the density of dry protein), the diameter does not really correspond to that of the hydrated protein in the aqueous clot environment; and the degree of lateral swelling by hydration cannot be determined from $\mathcal{D}$. Carr et al. showed that this ambiguity can be avoided by calculating an alternative measure of thickness, ν, which is the number of fibrin monomer units lying side by side in a fibrous element. In our notation,

$$\nu = N_A l \pi \mathcal{D} c / 4kM, \tag{8}$$

where N_A is Avogadro's number, l the length of a monomer unit (taken as 450 Å), and M the fibrin monomer molecular weight (330 000); this is independent of any assumption about hydration.

In table 1, values of ν are listed for clots of various degrees of coarseness (adjusted primarily by varying the thrombin concentration). They do not include any extremely fine clots (such as formed at higher pH), but the value of ν is as low as 4 which is close to the minimum value of 2 expected for the staggered over-

Table 1
Average fiber thickness of various clots (pH 7.5, μ 0.15)

F' gen [a] g/ℓ	Thrombin u/ml	$\mathcal{D}$ ($\times 10^{10}$)	ν
3.1	6	0.34	6.7
5.9	6	0.19	7.2
6.3	6	0.19	7.7
9.4	6	0.074	4.4
12.5	6	0.051	4.0
4.8	0.8	1.94	59
8.6	0.8	0.46	25
13.6	0.8	0.077	6.7
9.3	0.6	0.083	49
9.3 [b]	0.6	7.21	426
9.3	3.5	0.38	22
9.3 [c]	3.5	2.94	174
9.3 [d]	3.5	1.40	83

[a] The difference between fibrinogen concentration before clotting and fibrin concentration after release of fibrinopeptides has been ignored in the discussion.
[b] With 0.004 M calcium ion; μ = 0.16.
[c] With 0.006 M calcium ion; μ = 0.17.
[d] With 0.004 M calcium ion and fibrin stabilizing factor; μ = 0.16.

lapping pattern postulated for the first stage of polymerization into protofibrils [6–8]. For the coarsest clots, the values are much greater. The results are consistent with those of Carr et al. [2], who found ν near 5 for fine and about 500 for coarse clots but did not have intermediate values from permeation measurements; their concentrations were considerably lower than ours.

In fig. 4, ν is plotted against the concentration of the fibrinogen solution clotted for two series of clots, coarse and fine. For the coarse series ν is approximately proportional to c^{-2}. For the fine series, the data are somewhat scattered but are consistent with the conclusion from a more reliable earlier analysis of all the frequency-dependent data for G'_a and G''_a that $\mathcal{D}$ is proportional to $c^{-1.5}$ and hence ν is proportional to $c^{-0.5}$. Hermans and associates suggested that the opposite dependence on concentration might be expected from equilibrium considerations. Actually, they found ν to decrease with increasing concentration for a series of fine clots and to increase with concentration for a series of exceedingly dilute coarse clots. It is likely that the extent of lateral aggregation is controlled by kinetics rather than equilibrium (as evidenced by the strong effect of thrombin in fig. 4 and in fig. 1 of ref. [1]), except perhaps at very low concentrations.

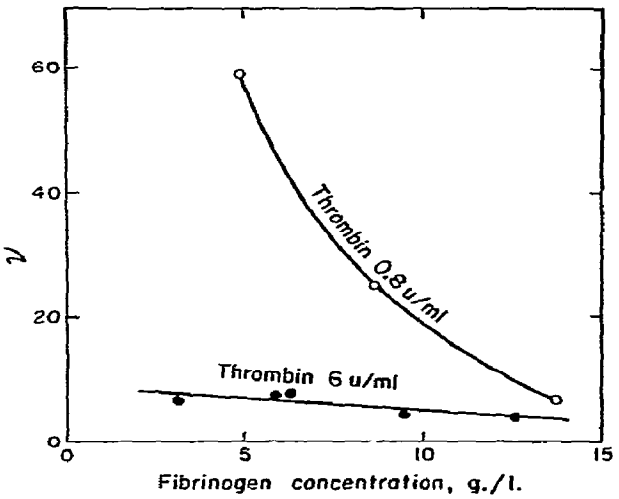

Fig. 4. Average number of monomer units per fiber cross-section, ν, plotted against fibrinogen concentration, for two thrombin concentrations as indicated; pH 7.5, μ 0.15.

It has been suggested [9,10] that the fine clot is a metastable structure; the tendency to lateral aggregation of protofibrils, which presumably increases with their length, is blocked by steric hindrances when the lengthening protofibrils interpenetrate at random angles. Such blockage would become more effective the higher the fibrin concentration and may be the source of the concentration dependence of thickness seen in fig. 4. A more detailed analysis of kinetics would be necessary to explain the difference in exponent for the fine and coarse limiting types, and the change to a positive exponent as observed by Hermans at extremely low concentrations.

In table 1 it is also evident that the presence of 0.004 M calcium ion increases ν substantially whether or not additional fibrin stabilizing factor is present. This effect can also be seen in the opacity [1]. Calcium also enhances and increases the rate of development of clot rigidity, as shown by Shen et al. [11].

Acknowledgement

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