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RHEOLOGY OF FIBRIN CLOTS. V. SHEAR MODULUS, CREEP, AND CREEP RECOVERY OF FINE UNLIGATED CLOTS

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Creep and creep recovery in small shearing deformations have been studied in fibrin clots at pH 8.5 and ionic strength 0.45, where the fine, transparent clot is formed with very little lateral aggregation of protofibrils. The initial shear modulus G_1 was measured 25 s after deformation on clots aged long enough for complete development of structure. For both human and bovine fibrin, the data were approximately described by $\log G_1 = 1.45 + 1.90 \log c$, where c is concentration in g/l and G_1 is in dyn/cm^2, over a range of c from 4 to 13 g/l. For bovine clots with completely developed structure, creep and creep recovery showed substantial irrecoverable deformation but the differential modulus G_Δ measured at intervals agreed with G_1 and did not change during the course of the experiment; it also agreed with the value calculated from the initial recovery after removal of stress. Moreover, several tests showed that the course of recovery conformed closely to the Boltzmann superposition principle. Thus the irrecoverable strain was associated with a structural rearrangement which caused no permanent damage. The irrecoverable deformation relative to the initial deformation was proportional to the elapsed time during creep in the early stages with a proportionality constant that decreased somewhat with increasing clot age prior to imposition of stress; it corresponded to a pseudo-viscosity of the order of 10^7 poise. However, the irrecoverable deformation does not represent viscous flow and appears to approach a limiting value at long times. Experiments on clots without completely developed structure, i.e., with imposition of stress at an earlier clot age, showed an increase in the differential modulus G_Δ during creep. The irrecoverable deformation was greater and a portion of it could be attributed to the balance between two structures formed in the unstrained and strained states. However, unlike the case of *ligated* clots strained before complete development of structure, where the irrecoverable deformation is entirely due to a two-structure balance, there is also a contribution from structural rearrangement. Experiments with reverse creep and creep recovery showed that the structural rearrangement is symmetrical with respect to direction of deformation. The interpretation of these results in terms of clot structure and internal motions of protofibrils is discussed.

1. Introduction

Viscoelastic properties of fibrin clots have been described in previous papers of this series [1,2] with emphasis on the shear modulus measured at short times where it is essentially time-independent and on the creep behavior under constant stress over long time periods followed by creep recovery. Different behavior is observed depending on the degree of lateral aggregation of the protofibrils which are the initial product of polymerization of the fibrin monomer, the extremes being the "fine" transparent clot formed at high pH and ionic strength in which the fiber diameter corresponds to not much more than that of a single protofibril and the "coarse" opaque clot in which it is hundreds of times thicker [3–6]. The mechanical properties also depend on whether the noncovalently bound junctions between the monomer units have been ligated, i.e., reinforced with covalent bonds introduced by fibrinoligase (Factor XIIIa). Properties of coarse ligated [1], coarse unligated [2], and fine ligated [2] clots have been described with only a few data for fine unligated.

In the present paper, the properties of the fine unligated clot are examined in more detail. This structure, though not physiological, is perhaps the simplest because of the absence of lateral aggregation. The protofibrils are presumably formed by staggered overlapping of fibrin monomer units to give a linear array with twice the monomeric mass per unit length [7], as evidenced in particular by the repeat distance of 225 Å observed in electron microscopy [8] and small-angle X-ray scattering [8–10], which is half the monomer length, and also by the mass per unit length

of stabilized oligomers obtained from light scattering [11,12]; although simple end-to-end junctions of the monomer units have also been proposed. When there is no ligation, creep under constant stress followed by creep recovery reveals a non-recoverable, permanent deformation whose magnitude depends on the creep duration and the age of the clot at the beginning of creep. Measurements of differential elastic modulus and of reverse creep and creep recovery permit conclusions to be drawn concerning the effects of the time-dependent deformation on clot structure. In the experiments reported here, the shear strains are always very small, generally less than 3%. The time-dependent behavior of fine unligated clots in large strains is quite different and will be reported subsequently.

2. Materials

Human fibrinogen, grade L, was obtained from Kabi AB, Stockholm. It was dissolved in buffer of pH 8.5 and ionic strength 0.45 of which 0.40 was contributed by sodium chloride and 0.05 by trishydroxymethylaminomethane (tris), then dialyzed against a large volume of the same buffer overnight with one change. Bovine fibrinogen, Fraction I with clottability 65%, was obtained from Miles Laboratories and purified usually by ethanol precipitation to obtain Fraction I-4 as described by Blombäck [13]. Protein concentration was determined spectrophotometrically by absorption at 280 nm with correction for scattering at 320 nm. The clottability of both human and bovine preparations was generally more than 92%, and the difference between fibrinogen and total protein concentrations was ignored. Bovine thrombin from Parke-Davis was dissolved in tris buffer at pH 7.5. ionic strength 0.15 and twice dialyzed against a large volume of the same buffer at 4°C. The activity was measured by standard methods [14], but the amounts used in experiments were determined primarily by the desired clotting times, usually between 10 and 20 min. The final pH and ionic strength were not significantly affected by the small volumes of thrombin solution used. Trasylol (proteolytic inhibitor) was obtained from FBA Pharmaceuticals. Materials for polyacrylamide gel electrophoresis (acrylamide with cross-linking comonomer, dithiothreitol, Coomassie Brilliant Blue dye) were obtained from Biorad Laboratories.

3. Methods

The clotting mixtures contained 4 to 13 g/l of fibrinogen, 2 units/ml of Trasylol (to inhibit plasmin in case of possible contamination), 1 mM ethylene diamine tetraacetate (EDTA, to sequester possible traces of calcium ion and insure absence of fibrinoligase activity in case of contamination by fibrin stabilizing factor, Factor XIII), and the appropriate concentration of thrombin. One portion was introduced before clotting into the Plazek torsion apparatus [1,15] and another was reserved for polyacrylamide gel electrophoresis after clotting. The electrophoresis of a solution solubilized by sodium dodecyl sulfate-urea and reduced by dithiothreitol was performed by conventional procedures [16] on both the clot and the original fibrinogen. Both always showed a small amount ($< 5\%$) of γ-γ ligation and no α-α ligation. The γ-γ ligation in the fibrinogen is attributed to a small amount of ligated dimer formed during the collection of the blood [17], seen also in agarose gel electrophoresis [18]. The fact that it is unchanged after clotting confirms that the clots are unligated.

In the Plazek apparatus, the disk-shaped clot is deformed in torsion, with a maximum shear strain of 2%. Previous studies have shown that this is well within the range of linear viscoelastic behavior [2,9,20]. To measure the initial compliance, J_1, the strain γ_1 is measured after the stress σ has been applied for 25 s; $J_1 = \gamma_1/\sigma$. Its reciprocal is the initial modulus, G_1. There is very little time dependence of the modulus (or compliance) of a fine unligated clot in the time range from 10^{-2} to 10^2 s [1]. To study creep, the strain is measured as a function of time, $\gamma(t)$, at constant stress, σ, and the creep compliance is expressed as $J(t) = \gamma(t)/\sigma$. In creep recovery, the stress is reduced to zero at time t_1 and the strain is measured as a function of time thereafter. In some experiments, a stress was subsequently imposed in the opposite direction for a reverse creep and creep recovery determination. At any point in the creep and creep recovery sequence, the differential compliance J_Δ can be obtained as the ratio between an incremental strain and an incremental stress measured 25 s after imposition of the incremental stress, which is then immediately removed. The reciprocal of J_Δ is the differential modulus G_Δ; the time profile for this kind of measurement is illustrated in figs. 5 and 6 of ref. [2].

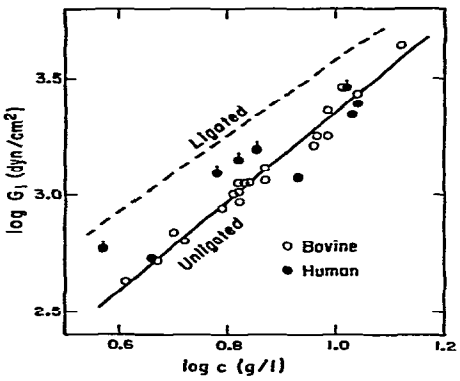

Fig. 1. Initial shear modulus (at 25 s) plotted logarithmically against fibrin concentration for fine clots. Points and solid line, unligated (tagged points from ref. [1]), slope = 1.9; dashed line, ligated human fibrin from ref. [2], slope = 1.6.

The modulus increases with the age of a fine unligated clot for several hours [2,19]. In the clots described here, there was very little change after 3 to 7 h, although between 7 and 24 h there is a difference in the irrecoverable deformation as will be seen. Measurements made after 7 h represent the properties of a completely developed clot structure. At shorter times, the creep and creep recovery behavior is different as will be shown.

4. Results

4.1. Dependence of initial modulus on fibrin concentration

The 25-s shear modulus G_1 ($= 1/J_1$) for fine unligated clots with completely developed structure is plotted logarithmically against fibrin concentration in fig. 1. The data for both bovine and human fibrin fall on a line with a slope of 1.9; it corresponds to the equation $\log G_1 = 1.45 + 1.90 \log c$ (G_1 in dyn/cm^2). Some points for human fibrin from an earlier publication [1] are included; they lie a little higher, and may reflect partial ligation since in that earlier work EDTA was not used and the ligation was not routinely monitored. (However, the lack of reproducibility in modulus for fibrinogens from different sources has been mentioned previously and stressed by Hermans and coworkers [20].) The line for fine ligated clots is reproduced from ref. [2]; it is higher by a factor of 1.6 to 2 and has a slope of 1.6. Measurements by Hermans and co-workers [20] on human fibrin in a similar concentration range appear to have a slope of about 1.8; however, their clotting conditions probably correspond to coarse rather than fine clots, and the magnitude of the modulus is somewhat higher. At lower fibrin concentrations, they found the slope to be 2.0 [21]. There appears to be no significant difference between bovine and human fibrin.

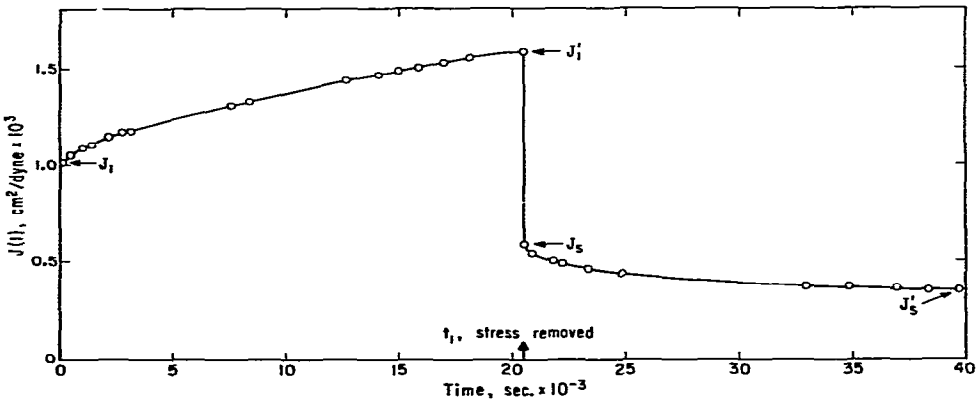

Fig. 2. Creep compliance and creep recovery plotted linearly against time for fine unligated clot (bovine); fibrin 6.54 g/l, pH 8.5, μ 0.45, clotting time 16.5 min, age at start of creep 24 h, irrecoverable relative to initial deformation 0.36 (expt. 159).

4.2. Creep and creep recovery of clots with completely developed structure

The creep compliance of a clot with completely developed structure (age 24 h at start of creep) including creep recovery after 2.05×10^4 s is plotted in fig.2. The fibrin concentration was 6.54 g/l. On the graph are indicated the initial compliance, $J_1 = 1/G_1$; the final compliance at the end of the creep interval, J_1'; the initial compliance 25 s after start of recovery, J_s; and the final compliance, J_s', proportional to the irrecoverable deformation. In previous studies of fine ligated clots [2], very little creep was observed over a period of 10^4 s (J_1' only slightly greater than J_1) and recovery was almost complete (J_s' smaller than J_1 or J_1' by more than two orders of magnitude). For this unligated clot, there is substantial creep and also a large permanent (irrecoverable) deformation J_s'. However, the structure of the clot has undergone no permanent damage. There are several sources of evidence for this statement.

In table 1, compliance data are given for the clot of fig. 2 and eleven others with various fibrin concentrations, ages, and creep durations (t_1). In addition to the initial compliance J_1, for several clots a differential compliance J_Δ was measured by incremental stress at time t_1 just before stress removal. Finally, the removal of stress at t_1 is equivalent to applying a negative stress and so the difference $J_1' - J_s$ also represents a differential compliance which is tabulated for all clots. All three of these values agree closely, showing that although a large proportion of the deformation is irrecoverable the structure has not been damaged.

Moreover, the entire course of the recovery can be predicted from the Boltzmann superposition principle, which implies no structural changes. In a pair of experiments on duplicate clots, (a) stress is removed at time t_1 and recovery is followed; (b) the creep is followed to times considerably longer than t_1. The compliance during recovery ($t > t_1$) is then calculated as

$$J_a(t) = J_b(t) - J_b(t - t_1). \quad (1)$$

An example of this test is shown in fig. 3 and the agreement is excellent. (Similar agreement has been found also for both fine and coarse ligated and coarse unligated clots previously [1,2] as long as the clot structure is completely developed before creep begins.) Thus, for the fine unligated clots, although the structure has rearranged under stress, there is no permanent damage for the small strains used here.

4.3. Dependence of irrecoverable deformation on creep duration

The final compliance after recovery, J_s', increases with creep duration and also decreases somewhat with the age of the clot even after the structure is completely established as evidenced by approximate constancy of modulus and the agreement of differential compliances as seen in table 1. However, it is difficult to compare various clots directly because of differences in fibrin concentration and other possible sources of irrepro-

Table 1
Creep and recovery data for clots with completely developed structure. Bovine fibrinogen; compliances in cm^2/dyne

Expt No.	Conc. g/l	Age h	t_1 s $\times 10^{-4}$	J_1 $\times 10^4$	J_Δ at t_1 $\times 10^4$	$J_1' - J_S$ $\times 10^4$	$u = J_S'/J_1$
137	7.36	7	0.72	7.71	7.54	7.44	0.31
136	7.42	7	1.40	8.34	7.93	8.09	0.73
135	9.44	9.2	0.72	4.38	4.28	4.26	0.26
140	4.09	15	8.0	23.4		23.4	0.67
141	13.2	18.5	2.0	2.31		2.28	0.41
166	10.0	24	0.72	2.94	3.01	2.85	0.10
159	6.54	24	2.0	9.86		9.95	0.36
167	8.28	24	3.42	4.22		4.27	0.61
199	8.15	24	10.0	4.48		4.60	0.77
200	5.01	24	10.0	13.45		13.31	0.96
201	6.41	24	10.0	7.30		7.40	0.70
173	11.3	48	0.10	2.54		2.55	0.03

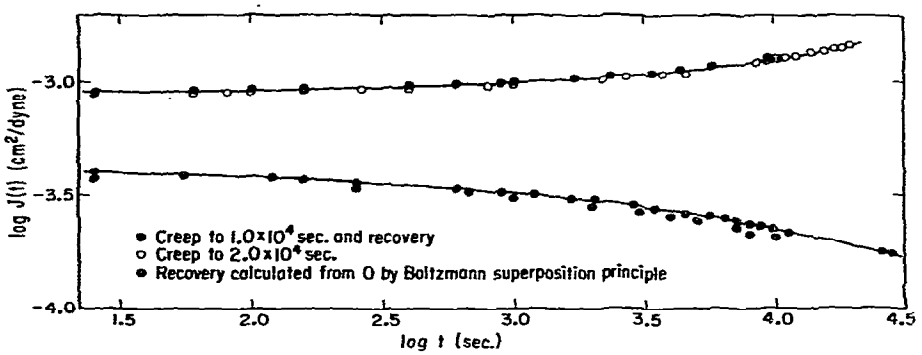

Fig. 3. Creep compliance and creep recovery compliance for a fine unligated clot plotted logarithmically with test of Boltzmann superposition principle by a pair of experiments. Fibrin (bovine) 6.8 g/l, pH 8.5, μ 0.45, clotting time 18 min, age at start of creep 24 h, permanent relative to initial deformation 0.135 (expts. 145, 146). For the recovery curve, time is counted from removal of stress, i.e., $t - t_1$.

ducibility. Use of the irrecoverable deformation relative to the initial deformation, as measured by the ratio $J_s'/J_1 \equiv u$, eliminates the effects of these differences; it is tabulated in the last column of table 1, and plotted against creep duration in fig. 4.

At times up to several hours, the relative irrecoverable deformation is directly proportional to the creep duration; it is approximately 2.5 times as great for clots aged 7–9 h as for clots aged at least 18 h. However, at longer times the irrecoverable deformation appears to reach a maximum. In the experiments shown here, this maximum irrecoverable strain is about 0.02, so we are looking at very small strains in the range of direct proportionality.

In conventional creep experiments on linear viscoelastic materials, a steady-flow viscosity η_0 can be calculated from the relation $\eta_0 = t_1/J_s'$. In the present system, there is clearly no steady flow, both because of the departure from linearity in fig. 4 at long times and because the creep $J(t)$ does not become a linear function of time in plots such as fig. 2 even for very long creep durations. However, estimates of a pseudo-viscosity can be made from measurements in the linear region of fig. 4. For example, for experiment 159 (fig. 2), the above relation gives $\eta_0 = 5.6 \times 10^7$ poise. Curiously, this is also the final slope of the creep curve in fig. 2 even though at t_1 this slope has not become constant. Similar agreement has been found for these two calculations of pseudo-viscosity from data of numerous clots both with and without completely developed structures [19].

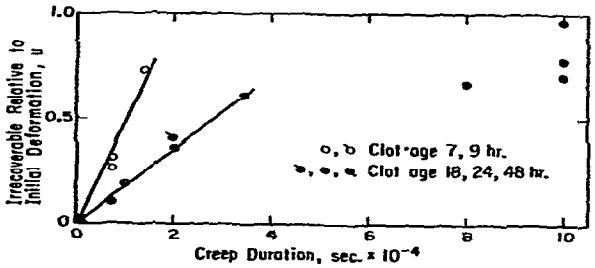

Fig. 4. Relative irrecoverable deformation u plotted against duration of creep for fine unligated clots (bovine) with completely developed structure, pH 8.5, μ 0.45, other data given in table 1.

4.4. Creep and creep recovery of clots with incompletely developed structure

The creep compliances of two clots with almost completely and with incompletely developed structures are compared in fig. 5. For the former, the differential modulus increases only very slightly during the experiment. For the latter, there is a substantial increase during the creep portion, showing that additional structure is formed while the clot is in a strained state.

In fine ligated clots, there is no permanent deformation from structural rearrangement as described in the preceding section, but a permanent deformation

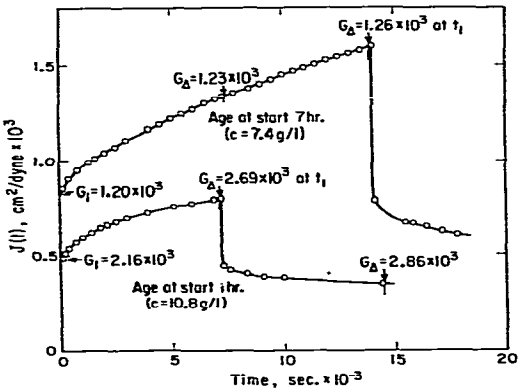

Fig. 5. Creep compliance and creep recovery plotted linearly against time for fine unligated clots (bovine) with age at start of creep and fibrin concentration (c) as shown; pH 8.5, μ 0.45. Age 1 h, clotting time 17 min (expt. 133); age 7 h, clotting time 14 min (expt. 136). Initial moduli G_1 and differential moduli G_Δ are shown.

is observed for an entirely different reason if the structure is not completely developed at the beginning of creep. The additional structure formed in the strained state is isotropic in the deformed shape and tends to maintain that shape after removal of stress; a state of ease is finally reached in which the original and additional structures pull in opposite directions and balance. For such clots, the permanent deformation calculated from a two-structure model agrees very well with experiment [2]. For the unligated clots described here, subjected to creep before the structure is completely developed, the permanent deformation is due to *two* causes: structural rearrangement as treated in the preceding section plus competition between two structures with different isotropic states.

For fine ligated clots, the prediction of the two-structure model can be calculated as follows. The modulus of the second structure can be obtained as $G_2 = G_\Delta - G_1$ where G_Δ is measured at t_1 just before removal of the stress. (Alternatively, G_Δ should be equal to $(J_1' - J_s)^{-1}$ as shown in table 1.) In the state of ease $\gamma_s' = J_s'\sigma$, the first structure has a strain γ_s'. The second structure is actually made up of elements with different strains because it was formed while the strain was changing from $J_1\sigma$ to $J_1'\sigma$; however, most of it was formed in the early stages of creep and, to avoid a complicated integration for which the data are not available, it is assumed that the strain of the second structure is $\gamma_1 - \gamma_s' = (J_1 - J_s')\sigma$. (For the fine ligated clots treated previously, J_1 and J_1' differ so little that there is no ambiguity.) In the state of ease, the stresses balance:

$$G_1\gamma_s' = G_2(\gamma_1 - \gamma_s') \tag{2}$$

and the calculated value of $J_s'/J_1 = \gamma_s'/\gamma_1$ for the two-structure model is then

$$u_{2s} = G_2/(G_1 + G_2). \tag{3}$$

Data for a number of clots with incompletely developed structures are given in table 2. Only clots with age 1.5 h or less are clearly in this category. (At 3 h, the data for several clots were not reproducible, some

Table 2
Creep and recovery data for clots with incompletely developed structure. Bovine fibrinogen; compliances in cm²/dyne

Expt No.	Conc. g/l	Age h	t_1 s×10⁻⁴	J_1 ×10⁴	J_Δ at t_1 ×10⁴	$J_1'-J_s$ ×10⁴	u	u_{2s}	$u-u_{2s}$
207	8.28	0.75	10.0	5.08	3.55	3.1	1.48	0.30	1.18
211	8.34	0.75	10.0	9.27	4.70	4.59	1.36	0.49	0.87
133	10.8	1.00	0.72	4.63	3.71	3.53	0.76	0.20	0.56
212	6.82	1.25	0.72	4.27	3.47	3.7	1.44	0.19	1.25
208	8.97	1.25	10.0	4.68	3.71	3.4	1.56	0.21	1.35
147	6.82	1.5	0.72	11.6	10.2	9.6	0.46	0.12	0.34
206	6.82	3.0 [a]	10.0	6.58	3.93	4.0	0.97	0.40	0.57
214	7.90	3.0 [a]	10.0	4.59	4.23	4.5	1.46 [b]	0.08	1.38 [b]
214R [c]	7.90	32.2	10.0	4.52	4.51	4.7	1.40	<0.01	1.40

[a] Several other experiments on clots with age 3 h showed variable degrees of completion of structure.
[b] Final value not available. [c] Reverse creep and recovery.

having fully developed structure as indicated by equality of J_1, J_Δ at t_1, and $J_1' - J_s$, and others not; the differences were not correlated with clotting times, which were all between 10 and 15 min.)

Now, J_Δ at t_1 is smaller than J_1, showing that additional structure has developed during the creep. The values of J_Δ and $J_1' - J_s$ agree quite well, as they should since they both represent determinations of differential compliance at t_1; $J_\Delta^{-1} = G_1 + G_2$. The modulus of the second structure can be obtained as $G_2 = J_\Delta^{-1} - J_1^{-1}$. The permanent relative to initial deformation to be expected from the two-structure model, u_{2s}, is then calculated from eq. (3). It is significant but it is always considerably smaller than the observed value u, so the major part of the permanent deformation comes from structural rearrangement. The data are insufficient to determine the dependence of the difference, $u - u_{2s}$, on creep duration by constructing a graph similar to fig. 4. However, it is evident from the values at $t_1 = 0.72$ that for clots aged 1 or 1.25 h the permanent deformation is accomplished in a much shorter time than for the clots with completely developed structure, and possibly a maximum value of $u - u_{2s}$ is reached rather quickly.

4.5. Reverse creep and recovery

As a further demonstration that the structural rearrangement responsible for irrecoverable deformation causes no structural damage, several experiments were performed in which creep and brief creep recovery, ending with a permanent deformation $\gamma_s' = \sigma J_1'$ (not the final value), were followed by imposition of stress $-\sigma$ in the opposite direction and a second creep and recovery. In the reverse experiment all strains were measured relative to γ_s' as zero. The profiles of stress and strain, including incremental changes to determine J_Δ at several points, are shown in fig. 6. The reverse creep is an accurate mirror image of the original creep and the values of J_Δ are nearly equal throughout as seen in the last two rows of table 2, ending with a value of 4.51×10^{-4} cm^2/dyne after the final recovery as obtained from $\Delta\gamma_3/\sigma_3$ on the profile. Thus the structural rearrangement is symmetrical for a reversal of deformation, and the structure remains undamaged throughout.

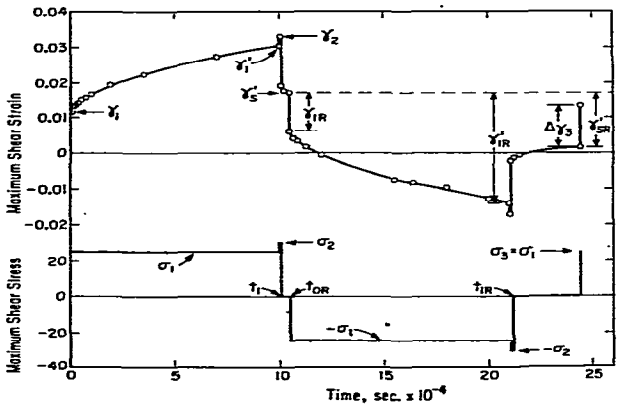

Fig. 6. Time profile for stress and strain in experiment with creep followed by creep recovery and then reverse creep and creep recovery with final brief stressing to determine differential modulus. Bovine fibrin 7.9 g/l, pH 8.5, μ 0.45, clotting time 14.5 min, age at start of creep 3 h (expt. 214).

5. Discussion

5.1. Dependence of initial modulus on fibrin concentration

The dependence of modulus on approximately the second power of concentration may be important in elucidating the molecular mechanism of elastic energy storage, which still remains in doubt. It has been postulated [2] that the fine clot is a random assembly of stiff rodlike protofibrils which are immobilized by steric interlocking and are not necessarily attached to each other by noncovalent (or, in the case of ligated clots, covalent) bonding. According to this view, the structure should be thermodynamically metastable and the protofibrils would tend to aggregate laterally to form a more ordered phase [22] if they were not prevented by steric interlocking. In fact, lateral aggregation occurs if the structure is broken up by sonication [23] and can even be induced by gentle mechanical manipulation with moderately large strains. Moreover, for given pH and ionic strength, there is less lateral aggregation during clot formation the more rapidly clotting takes place [6,24], as would be expected if it is inhibited by rapid protofibril growth.

In this concept, it has been suggested that elastic energy storage occurs at small strains through slight bending of the protofibrils. The elastic properties of an array of independent rods, interacting only by steric

repulsion and storing energy by bending, have been recently calculated on this basis by Doi and Kuzuu [25]. However, they predict a stress proportional to the fifth power of concentration instead of the second power observed. The difference may imply that the protofibrils adhere somewhat at points of contact, or that stretching rather than bending of the protofibrils is involved in elastic energy storage. Further work is necessary to clarify the elastic mechanism.

5.2. Development of structure with time

Three stages can be distinguished in the structural development of a fine unligated clot. First, oligomers are formed with an increase in light scattering which soon becomes insensitive to oligomer length, as found by Hantgan and Hermans [6]. The modulus of rigidity increases more slowly, and in our experiments requires of the order of 3 h to approach its limiting value, although these times are not reproducible. Somewhat more slowly still, the rate of irrecoverable deformation in a creep experiment diminishes with aging of the clot, as illustrated in fig. 4.

The early stages of oligomer formation in fine clots proceed in accordance with the kinetics of difunctional condensation polymerization, according to the deductions of Hantgan and Hermans [6], and a distribution of molecular lengths is formed. The elastic modulus of an interpenetrating array of rods should increase with average length until this is long enough so that each rod has many points of collision, beyond which the modulus is independent of length (as predicted for the model of Doi and Kuzuu [25]). However, if the achievement of irrecoverable deformation is associated with lengthwise motions of the rods to escape from their steric restraints as treated by Doi [26], this process should continue to become slower with increasing length; in fact, the Doi theory predicts a steady-flow viscosity which is proportional to the 6th power of length [26].

5.3. Mechanism of irrecoverable deformation

It seems plausible that the basic mechanism for irrecoverable deformation is structural rearrangement by sliding of rodlike protofibrils in a primarily axial direction to escape from their steric restraints as stated in the preceding paragraph. The rods would enter new constraints so the differential modulus G_Δ would remain constant and the structure would show no permanent damage. The Doi theory shows that this mechanism can give a steady flow viscosity corresponding to the linear portions of the plots in fig. 4. However, two additional facts must be accounted for: the irrecoverable deformation is almost completely eliminated by ligation, which in fine clots is almost exclusively γ-γ ligation; and it appears to reach a limiting value at long times instead of continuing with steady-state flow.

Two possible interpretations can be given for the effect of ligation. (a) Although the ligation is mostly γ-γ, an occasional α-α linkage prevents the sliding of protofibrils along their axes and thus eliminates irreversible deformation. (2) Except for very short oligomers, rupture of protofibrils by dissociation of noncovalently bound junctions between adjacent monomer units is a prerequisite for axial motion, and this is of course eliminated by γ-γ ligation. This is plausible in view of the expected very rapid decrease of rod mobility with increasing rod length. However, it is necessary to assume that the dissociated junctions join again in new positions to account for the absence of permanent damage. A few unhealed dissociations would not affect G_Δ, but would not be consistent with the exact agreement between forward and reverse creep and recovery in fig. 6, for example.

The limiting value of irrecoverable deformation seen in fig. 4 may be associated with a limit to the extent of axial motion even of shorter rod lengths formed by dissociation of junctions. The maximum irrecoverable train in these experiments was of the order of 0.02, and perhaps larger strains cannot be achieved without damage. In other experiments now in progress, larger strains cause large changes in the differential modulus [27].

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