Non-Newtonian Behaviour of Dilute DNA Solutions

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The decrease in viscosity of dilute solutions of DNA when subjected to shear rates in the range 0-200 sec^{-1} has been investigated with samples from a number of different sources. The nucleic acid molecule behaved as a coiled particle with a considerable degree of flexibility, and there was no evidence of any species differences in this respect. Samples of DNA with an intrinsic viscosity $[\eta] \geqslant 90$ dl/g showed a discrepancy between intrinsic viscosities measured in a rotating cylinder apparatus and in a capillary tube, which suggested that a shear-sensitive structure persisted in the dilute solutions of high molecular weight DNA.

The non-Newtonian behaviour of nucleic acid solutions has been investigated with conflicting results.[1, 2] The solutions were very dilute ($< 5 \times 10^{-5}$ g/ml), and sufficient sodium chloride was present to suppress electrostatic effects both inter- and intra-molecular. Such conditions approximate to the desired limit, viz., the behaviour of the individual particle. Thus, in the absence of any structure, the degree of departure of dilute solutions from Newtonian behaviour, judged by the fall in viscosity with increasing rate of shear, should be a function of the shape of the macromolecule and of its flexibility.

For models representing relatively rigid molecules such as T.M.V., the mathematical calculations are well established.[3] The situation for the class of flexible particles, which includes most synthetic and naturally occurring macromolecules, is much more complex, and only approximations have been made.[4-6] Eisenberg compared the viscous behaviour of thymus nucleic acid solutions with that expected for a coil-like particle with high impedance to the movement of chain segments. His nucleic acid seemed more rigid than this model would predict, whereas Hermans and Hermans found for various preparations of DNA that there was almost no non-Newtonian behaviour in dilute solutions up to $G \sim 40$ sec^{-1}.[2] All these investigations used nucleic acid of intrinsic viscosity *ca.* 60 dl/g. We have obtained results similar to those of Hermans and Hermans, which would suggest a considerable degree of flexibility of the molecule.

Undoubtedly, large molecules in solution can be ruptured by shear stresses. Bestul and others for hydrocarbon polymers in concentrated solution found a relationship between initial molecular weight and the damage produced by a given shear force.[7, 8] Similar investigations have been carried out with nucleic acids, at high dilutions and much lower rates of shear.[9, 10] If the nucleic acid molecule is relatively sensitive to shear forces, then it is likely to be damaged during isolation procedures. However, it is always difficult to eliminate the possibility that a loose structure in solution may be the sensitive point; the evidence furnished here suggests that this may be the case.

EXPERIMENTAL

ROTATING CYLINDER VISCOMETER

The instrument for measuring viscosities at low shear rates has been described elsewhere.[11] A variable speed drive has now been added in which an electronically governed motor is

connected directly to the outer cylinder and a range of speeds is obtained by a variable resistance. The motor and controller, supplied by Messrs. Hanovia Ltd., Slough gave a shear rate range from 0·5 to 40 sec^{-1}.

CAPILLARY VISCOMETER

In order to investigate shear dependence at shear rates greater than those in the Couette and less than those in the Ostwald capillary, a flat bed viscometer was constructed from 120 cm precision-bore tubing (0·5 mm diam.) joined at each end to two limbs, each 8 cm long, of 2 mm diam. precision tubing. The capillary was bent in such a way that the 2 mm tubes were parallel and could be arranged to lie on a flat table. The solution under test filled the capillary and up to half way along each wider limb. Pressure was then applied to one of the limbs and the time taken for either meniscus to move 5 cm was measured and compared with the corresponding time for the solvent. A 5-l. aspirator was connected to the viscometer and to a wide water-manometer (2·5 cm diam.), the other side of the viscometer and manometer being connected via a smaller buffer volume to the atmosphere. All the pressure-stabilizing apparatus was contained within a metal box to reduce the effect of draughts, and the manometer was viewed with a short focal length telescope. The difference in manometer levels could be read to 0·015 cm water pressure and when a timing error of 0·1 sec is allowed for, the error in relative viscosity η_r becomes $\pm 0{\cdot}7$ % at a shear-rate of 20 sec^{-1}, falling to $\pm 0{\cdot}35$ % at 180 sec^{-1}, the pressure errors being the more serious.

A series of measurements was made by timing the flow of solution, at a high pressure (*ca.* 25 cm water) and then reducing the pressure in stages by 1 cm to 10 cm, and then by 0·5 cm to *ca.* 2·5 cm. The flow was timed at each pressure, always in the same limb and in the same direction; a series of 30 measurements could be obtained in 2½-3 h.

The calculated relative viscosities were not corrected for non-Newtonian behaviour. Over the whole range of shear rates the fall in relative viscosity is such as to make the correction for any particular value less than *ca.* 1 % in η_r or G. By integrating the equations for streamline flow, allowing for the dependence of viscosity upon shear rate, then [12]

$$G_t = G_a(1-\tfrac{1}{4}\mathrm{d}\ln\eta_a/\mathrm{d}\ln\tau),$$

where G_t is the true, and G_a the apparent shear rate, τ is the shear stress, and η_a the apparent viscosity, obtained from the flow times of solvent and solution. Substituting experimental values for extreme conditions, viz., apparent relative viscosity of a 0·0013 % solution $\eta_a = 1{\cdot}07$ at a shear rate of 150 sec^{-1}, and $\mathrm{d}\eta_a/\mathrm{d}G = 4\times10^{-4}$ sec^{-1}, $G_t = 0{\cdot}985G_a$. The results for $G = 50$ sec^{-1} is $G_t = 0{\cdot}995G_a$.

PREPARATION OF SOLUTIONS

Nucleic acid solutions were made up to about 0·02 % in 10^{-3} N NaCl by shaking gently overnight at room temperature. The solutions were taken to 0·2 M in NaCl and centrifuged for 1½-2 h at 20,000 *g* to remove any gel. Dilutions were made with 0·2 M NaCl for viscosity measurements, and before experiments were carried out, the diluted solutions were filtered, under gravity only, through porosity 3 sintered glass to remove dust.

METHODS OF SHEARING THE SOLUTIONS

LOW VALUES OF SHEAR RATE

The effect of shear rates of 400 sec^{-1} was examined by passing about 10 ml solution at a suitable pressure through the capillary apparatus. The viscosity before and after treatment was measured in the Couette viscometer. The pressure applied to produce a shear rate G_K and the " time of residence " of the solution in the capillary are given by

$$p = (0{\cdot}12G)\mathrm{cm\ H_2O}; \quad t_R = 1{\cdot}28\times10^4/G_K \text{ sec.}$$

The value of G_K is the Kroepelin average, i.e., 2/3 of maximum value at the wall, and an applied pressure of 48 cm water, on the average, subjects each element of the solution to a shear rate of 400 sec^{-1} for 32 sec, during one passage through the capillary. Some solutions were also sheared by passing them through a wide capillary ($r = 0{\cdot}037$ cm, $l = 120$ cm) for which the time of residence was $t_R = 8{\cdot}7\times10^3/G_K$ sec.

HIGH SHEAR RATES

An apparatus was designed in which the test solution could be repeatedly passed through a short capillary at a high rate of flow, such that the average shear rate was 28,000 sec^{-1}. The residence time in capillary was 0·0306 sec, and the shear rate $G_K = 27{,}800$, $G_{max} = 41{,}700\ sec^{-1}$ (at the wall).

IRRADIATION OF SOLUTIONS

Doses were delivered by a 220 kV X-ray tube at a rate of 950 rad/min. The dose rate was measured by irradiating ferrous sulphate solution (10^{-3} M in 0·8 N H_2SO_4) under the same conditions and using a yield $G(Fe^{3+}) = 15$ per 100 eV.

PRECIPITATION

Nucleic acid solutions were made 2M in NaCl and added to an equal volume of 95 % ethanol. The precipitate was washed in 95 % ethanol and allowed to dissolve immediately in 10^{-3} M NaCl. Recovery was independent of initial DNA concentration and usually better than 95 %.

RESULTS

In fig. 1, 2 are given the curves between $(\ln \eta_r)/C$ and shear rate for a series of nucleic acid samples at low concentrations in 0·2 M NaCl. In fig. 3 are a series of curves expressing $(\ln \eta_r)/C$ as a function of C at zero shear rate; at a concentration of *ca.* 0·0015 % the value of $(\ln \eta_r)/C$ differs very little from the limit at zero concentration $[\eta]$. The dependence of $(\ln \eta_r)/C$ upon G for such a dilute solution has been taken to be the same as that for the intrinsic viscosity $[\eta]$. The effect of radiation upon dilute solutions of some samples of nucleic acids is seen in fig. 2. The concentration of DNA was 0·005 % and the shear dependence of viscosity was followed at a suitable lower concentration. The effect of X-irradiation is (*a*) to lower $(\ln \eta_r)/C$, (*b*) to increase the range of shear rates over which the viscosity is constant, and (*c*) to decrease the total fall in viscosity which occurs between the experimentally available limits of shear rate.

The permanent effects of high shear stress for some specimens of DNA are set out in table 1 and compared with the effect of precipitation. Although there are anomalies, in general shearing has little effect at concentrations of DNA between

TABLE 1

sample		initial value	after precipitation (>0·01 %)	after shearing 200 × at 28,000 sec^{-1} (>0·01 %)	after shearing and precipitation subsequently
V1	$[\eta]$	53	53 (98)	58	56 (96)
	k''	1·26	0·67	0·25	0·22
T2	$[\eta]$	37	30 (93)	35	37 (75)
	k''	0·77	0·50	0·74	0·88
HS 12	$[\eta]$	82	84 (93)	74	74 (94)
	k''	0·34	0·11	0·36	0·33
CSS 5B	(*a*) $[\eta]$	110	90 (98)	92	75 (93)
	(*b*) $[\eta]$	110	—	88	80 (93)

Figures in parentheses are percentage recoveries; V1, calf thymus; T2, calf thymus; HS 12, herring sperm; CSS 5B, salmon sperm.

$$k'' = \Delta\left(\frac{\ln \eta_r}{C}\right) \Big/ \Delta C[\eta]^2 = k' - \tfrac{1}{2}, \text{ where } k' = \text{Huggins' constant.}$$

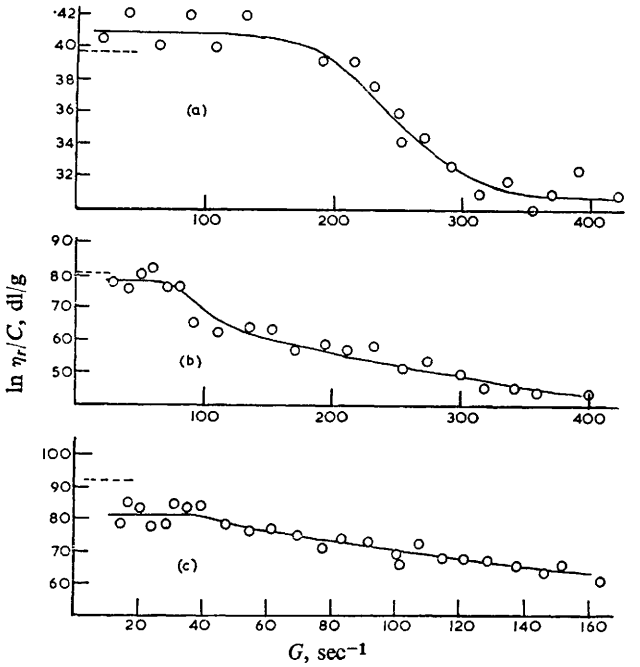

FIG. 1.—Effect of shear rate upon reduced viscosity of DNA samples in capillary. (*a*) 0·0025 % calf thymus (V8); (*b*) 0·00132 % Herring sperm (DNA 4); (*c*) 0·0016 *E. coli B/r*; - - - - indicates Couette value before shear.

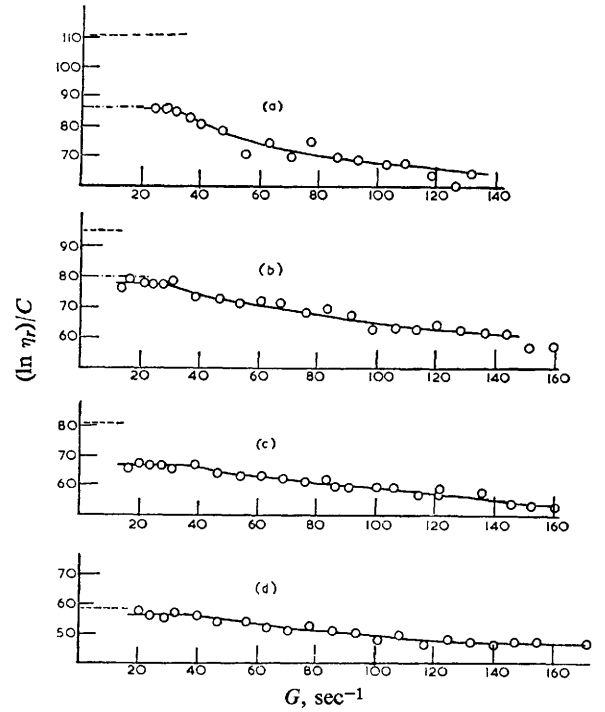

FIG. 2.—Effect of shear rate upon reduced viscosity of salmon sperm DNA (CSS 5B). (*a*) 0·0013 % unirradiated; (*b*) 0·0017 % after 190 rads to 0·005 % solution; (*c*) 0·0017 % after 380 rads to 0·005 % solution; (*d*) 0·0017 % after 760 rads to 0·005 % solution; - - - - Couette value before shear; —·—·— Couette value after shear at 400 sec^{-1}.

0·01 and 0·015 % with samples of $[\eta]<80$, but that above this value of $[\eta]$, some loss in viscosity occurs. Moreover, precipitation of the nucleic acid has almost the same effect upon $[\eta]$ as shearing. The last column of the table shows that precipitation of a sheared solution has no further effect on the viscosity in 3 out of the 4 preparations. There is no recovery of viscosity such as might be expected if a structure were reforming on subsequent solution of a precipitated sample. The values of k'' are decreased by precipitation and to a lesser extent by shearing, and this is particularly so where k'' is high, e.g., sample V1.

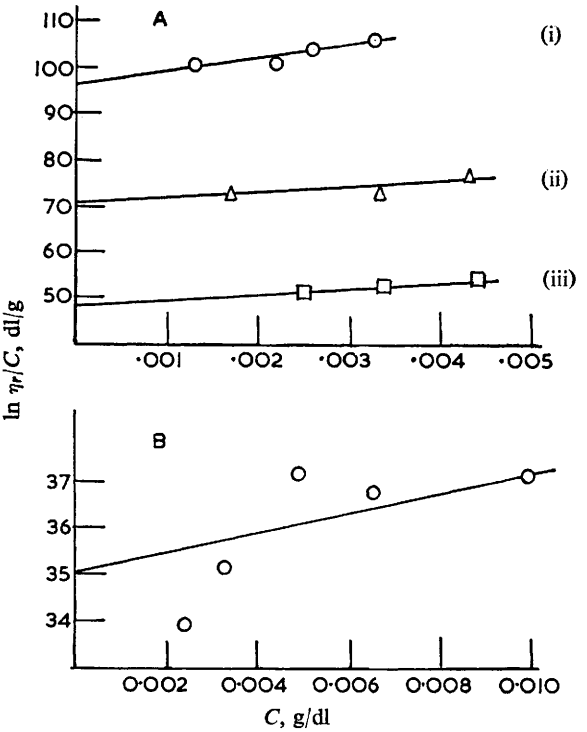

FIG. 3.—Relationship between reduced viscosity and concentration.

(A) Salmon sperm (1SS 2B): (i) unirradiated; (ii) after 380 rads to 0·02 % solution; (iii) after 760 rads to 0·02 % solution.

(B) Calf thymus (V8) unirradiated.

In fig. 1, 2 are plotted the Couette viscosities and the values obtained in the capillary apparatus. In some cases, a large discrepancy is evident, but in others agreement is good. In all, twelve specimens of DNA from different sources were examined; six of these showed agreement between the capillary and Couette at $G = 0$, whereas six showed a discrepancy. The former six had $[\eta]<80$ and the latter $[\eta]>90$. In an experiment on a high viscosity sample (ISS 2B; $[\eta] = 110$ dl/g), the capillary apparatus was filled such that the solution was subjected to a shear rate of less than 50 sec^{-1} and the value of $(\ln \eta_r)/C$ for G between 25 and 50 sec^{-1} agreed with the Couette val The conventional method of filling was by suction through the capillary and this procedure was calculated to develop values of $G = 400$ sec^{-1}. It is likely therefore that even such mild shearing causes damage when the concentration is low, and this was proved with all those specimens

which had $[\eta]>90$. One passage at a shear rate of 400 sec^{-1} and a concentration of 0·0012 % lowered the value of $[\eta]$, subsequently measured in the Couette, to about 80 dl/g, but further passages had no further effect. Moreover, the use of a wider capillary, 0·037 cm in radius, with the same average shear rate, caused no decrease even after a number of passages.

DISCUSSION

Coiled macromolecules fall into three classes from the point of view of non-Newtonian behaviour: (i) a completely random coil has perfect flexibility about the bond angles, and when placed in a field of velocity-gradient is alternately elongated and compressed during rotation.[13] Since the molecules can respond to this, no loss in viscosity results. (ii) A molecule which has limited rotation about the bonds cannot respond completely to stress on the different chain-segments, i.e., it has internal viscosity. The molecule becomes elongated, and although this tends to increase the hydrodynamic resistance to the flow of solvent, the orientation suffered by the particle due to its asymmetry has an overriding effect, and the viscosity decreases. (iii) A molecule which allows solvent to drain within its coils will lead to a further decrease in solution viscosity due to energy losses between the chain segments and the liquid surrounding them. The situation with DNA is represented by a combination of (ii) and (iii).

The fall in intrinsic viscosity $[\eta]_0$ with increasing shear-rate G may be represented as a expansion in G,

$$[\eta]_G = [\eta]_0(1-aG^2+bG^4-\ldots),$$

and all treatments of the problem involve modifications of this equation. Kuhn and Kuhn calculated a and b for a coiled molecule in which the internal viscosity was high, and together with the expression for the rotatory diffusion coefficient developed by Kuhn, Kuhn and Büchner [14] and used by Eisenberg for DNA,[1] this gives

$$[\eta]_G = [\eta]_0\left(1-\frac{0{\cdot}71\eta_0^2M^2[\eta]_0^2G^2}{R^2T^2}+\ldots\right),$$

where $[\eta]_G$, $[\eta]_0$ are the intrinsic viscosities of the solute at G sec^{-1} and zero shear-rate respectively, M is the molecular weight of the solute, and η_0 is the solvent viscosity. The same relationship is given by Cerf,[6a] but with a different constant in place of 0·71. However, Cerf has also given an alternative expression:

$$[\eta]_G = [\eta]_0\left(1-\frac{\gamma\eta_0^2M^2[\eta]_0G^2}{R^2T^2}+\ldots\right),$$

where $[\eta]_0$ is involved and not $[\eta]_0^2$. γ is a numerical constant. This latter equation was derived for a solute of low internal viscosity, and Cerf found that the behaviour of various hydrocarbon polymers was well expressed by this equation [6b].

In general, we may write

$$[\eta]_G-[\eta]_0 = -\frac{\gamma\eta_0^2M^2[\eta]_0^\beta G^2}{R^2T^2}+\ldots\ (\beta=2 \text{ or } 3);$$

assuming that M is proportional to $[\eta]_0$, which has been found for many samples of DNA,[15, 16]

$$([\eta]_0-[\eta]_G)/G^2 = K[\eta]_0^\alpha-\ldots, \text{ where } K=\gamma'\eta_0^2/R^2T^2.$$

The slope of the line between viscosity decrease and G^2, when expressed in terms of $[\eta]_0$, will then show whether α is nearer to 4 (for a soft coil) or 5 (for a stiff coil).

In practice, although the viscosity does not immediately decrease as G increases, a linear region is observed over a limited range of G^2 when $[\eta]_0-[\eta]_G$ is plotted against G^2 (fig. 4), until the terms in G^4, etc., become important.

When the slope of the linear portion is plotted logarithmically against $[\eta]_0$, the results fall on a straight line of slope $3{\cdot}1\pm0{\cdot}4$ (fig. 5). Two conclusions may be drawn from this plot. (i) The exponent shows that the DNA particles tend to

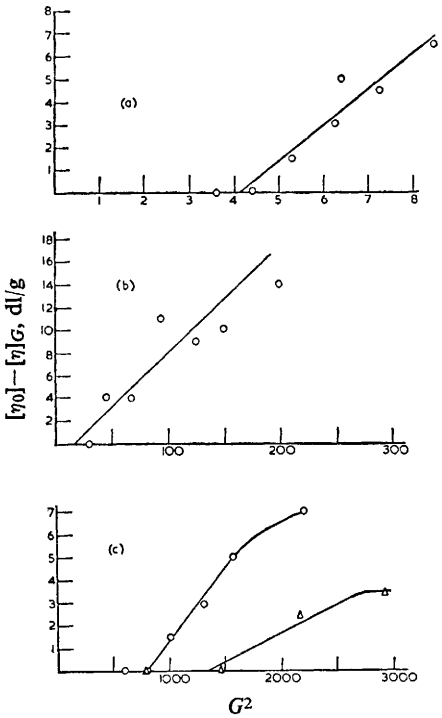

FIG. 4.—Relationship between $[\eta]_0-[\eta]_G$ and G^2.

(*a*) calf thymus (V8); (*b*) phage; (*c*) salmon sperm (CSS 5B): ○, unirradiated; △ after 380 rads to 0·005 % solution.

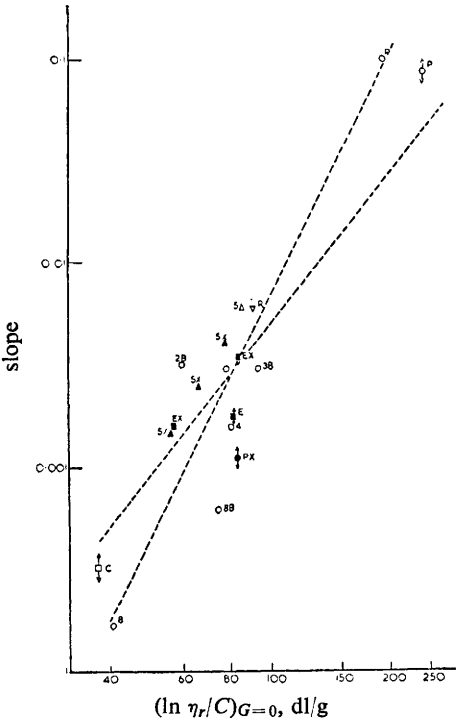

FIG. 5.—Relationship between slope of $[\eta]_0-[\eta]_G$ against G^2 curve and $[\eta]_0$. ($[\eta]_0$ assumed to be $\simeq(\ln \eta_r)/C$ at low concentration).

P, phage; CSS 2B, 3B, 5B, 8B, salmon sperm; R, rat thymus; 4, herring sperm; V8, calf thymus; C, Crocker tumour; E, *E. coli B/r* (irradiated *in vivo*).

In fig. 5 irradiated samples are designated × and have filled symbols. The dashed lines express the 95 % confidence limits of the best slope.

be " soft " (exponent = 4). (ii) The irradiated samples fall in a line with the native DNA, i.e., no increase in flexibility is produced by irradiation. This supports earlier evidence from sedimentation and viscosity studies.[17]

The initial flat portion of the $[\eta]_0-[\eta]_G$ against G^2 plot is hard to explain. There would appear to be a value of G below which behaviour is Newtonian and above which it is characteristic of particles oriented by flow. It is possible that for flexible particles, the effects of orientation and deformation are balanced over a limited range of shear stress. At some point the deformation reaches a limit. However, this cannot result from complete extension since this would be greater for the larger particles, and the " breakaway " point would also be greater. It is more likely

that deformation ceases because of resistance to further change in shape resulting from intramolecular forces.

It is uncertain what interpretation to put upon the lack of agreement between Couette and capillary viscosity for samples where $[\eta]>90$ dl/g. Since one passage of a 0·001-0·002 % solution of DNA through the capillary is sufficient to remove the difference between these values, the phenomenon is probably a critical one. The radius of the capillary is also critical. These facts suggest that a delicate structure is being broken down, rather than that DNA molecules themselves are being sheared. Davison *et al.*[10] have calculated that considerable shear forces are required to break a DNA molecule, unless the molecular weight is very high. The only evidence that back-bone breakage might be involved is rather circumstantial. Precipitated DNA, having suffered a fall in $[\eta]$ does not recover to its former value on redissolving, nor does a sheared solution ever "recover". Also the sharp change in behaviour observed between viscosities of 80 and 90 dl/g is more difficult to explain if intermolecular interaction is the critical factor rather than molecular size. On balance, it is probable that the mild shearing leads to a disentangling in a solution so dilute that intermolecular forces are not important. A higher concentration of DNA would be self-protective with respect to shear on this basis, and this is found in practice.

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