STUDIES ON THE SONIC DEGRADATION OF DEOXYRIBONUCLEIC ACID

DAVID FREIFELDER *and* PETER F. DAVISON
From the Department of Biology, Massachusetts Institute of Technology, Cambridge

ABSTRACT T7 DNA was partially degraded by x-rays, DNAase, and sonic irradiation. The molecular weight distributions were calculated from sedimentation velocity studies on the resulting preparations. Comparison with the theoretical curve derived by Montroll and Simha showed that the first two degradative methods act grossly at random, whereas sonication is a non-random process resulting in the preferential halving of the DNA molecules in solution.

INTRODUCTION

The purification and properties of T7 coliphage and of the DNA isolated from it have been described recently (1, 2). For physical and chemical experiments DNA[1] extracted from viruses offers several advantages over the DNA extracted from larger organisms (*e.g.*, simple extraction and purification, and low polydispersity). Bacteriophage T7 DNA has added advantages: (*a*) low shear sensitivity, (*b*) no unusual bases (such as glucosylated 5-hydroxymethycytosine in the DNA of T-even bacteriophages), (*c*) high purity (Resistance of the phage to osmotic shock allows it to be purified by cesium chloride density gradient centrifugation.), (*d*) homogeneity with respect to molecular weight.

A major difficulty involved in the interpretation of physical experiments on the DNA isolated by customary techniques from animal and bacterial sources arises from the broad polydispersity of these samples. When virus DNA is used for such experiments, this difficulty can be obviated if precautions are taken to prevent mechanical or enzymatic degradation of the molecules.

This paper describes the employment of T7 phage DNA as a test substance to examine the mechanism of sonic degradation. Montroll and Simha (3) have calculated the molecular weight distributions to be expected from the application of a

[1] Abbreviations used in this paper are as follows:—
DNA = deoxyribonucleic acid
DNAase = deoxyribonuclease
$s_{20,w}$ = sedimentation coefficient in Svedbergs corrected to the buoyancy and viscosity of water at 20°C

random degradative process to a monodisperse polymer. By comparing the molecular weight distribution of T7 DNA degraded by sonic irradiation (sonication)—using the empirical relationship (determined by Doty, *et al.* (4)) between the sedimentation coefficient, $s_{20,w}$, and the molecular weight of DNA—we have shown that sonication is not a random degradative process but proceeds by a mechanism analogous to hydrodynamic shear degradation (5–7). In contrast, the processes of degradation of DNA by x-rays and by DNAase *are* grossly compatible with random bond rupture.

EXPERIMENTAL TECHNIQUES

1. *Preparation of the Phage and the DNA.* Phage T7 (Luria) were grown and purified as described elsewhere (2). The DNA was prepared by the phenol method of Mandell and Hershey (8).

2. *Ultracentrifugation.* Ultracentrifugation was performed in the Spinco Model E ultracentrifuge at 33,450 RPM with a 30 mm Kel-F cell and ultraviolet optics. All solutions were centrifuged at a DNA concentration of 20 μgm per ml in M NaCl. Photographic plates were scanned with a Joyce-Loebl recording microdensitometer and the sedimentation distribution diagrams were calculated from these traces.

3. *Sonic Degradation.* Solutions of DNA at 40 μgm/ml in 0.01 M NaCl were placed in polyethylene vials which were then immersed in water in the cavity of a 10 kc Raytheon Model DF 101 sonic oscillator and irradiated for periods ranging from a few seconds to a few hours at 50 per cent power. This intensity is sufficient to produce a break in each molecule in about 15 seconds.

4. *Shearing.* Shearing was accomplished by passing the solutions rapidly and repeatedly through a No. 27 hypodermic needle.

5. *X-Irradiation.* Solutions of DNA at 40 μgm/ml in 0.01 M NaCl were x-irradiated at a dose rate of 12800 roentgens/minute from a Phillips Möd 150/Be x-ray tube with a beryllium window and ½ mm Al filtration; the tube was run at 150 kv and 12 milliamperes.

6. *DNAase Digestion.* Approximately 0.001 ml of a freshly prepared solution of DNAase (0.02 mg/ml.) (Nutritional Biochemical) was added to DNA at 40 μgm/ml in 0.01 M NaCl and 0.001 M $MgCl_2$ at 37°C. Enzyme action was stopped after a few seconds by chilling in ice water and adding an equal volume of 2 M NaCl. In M NaCl the enzymatic hydrolysis was negligible over ordinary time periods.

THEORY

Montroll and Simha (3) considered an initially monodisperse solution of N molecules each comprising $p + 1$ monomers linked by p equally vulnerable bonds. They showed that the application of a random degradative process which breaks a fraction α of the Np links in the polymer solution will produce a population of molecules in which a fraction $F_t(p, \alpha)$ of monomeric elements is involved in t-mers (i.e., molecules containing t monomers) where

$$F_t(p, \alpha) = \frac{\alpha t(1 - \alpha)^{t-1}}{p + 1} [2 + (p - t)\alpha] \qquad t \leq p \tag{1}$$

and

$$F_{p+1}(p, \alpha) = (1 - \alpha)^p$$

Since the values of p are integers, the molecular weight integral distribution is obtained by direct summation of (1) where

$$\sum_{t=1}^{q} F_t(p, \alpha)$$

is the weight fraction of the polymer in which fraction the molecules each contain q or fewer monomers. Substituting $x = 1 - \alpha$

$$\frac{p+1}{\alpha} \sum_{t=1}^{q} F_t(p, \alpha) = \sum_{t=1}^{q} t x^{t-1}[2 + (p - t)\alpha]$$

$$= (2 + p\alpha) \sum_{t=1}^{q} t x^{t-1} - \alpha \sum_{t=1}^{q} t^2 x^{t-1}$$

$$= (2 + p\alpha) \sum_{t=1}^{q} \frac{d}{dx} x^t - \alpha \sum_{t=1}^{q} [t(t-1)x^{t-2}x + t x^{t-1}]$$

By interchanging summation and differentiation and using the summation of the geometrical progression, then

$$\sum_{t=1}^{q} F_t(\alpha, p) = \frac{\alpha}{p+1} \left\{ (2 + (p-1)\alpha) \frac{d}{dx} \left(\frac{1 - x^{q+1}}{1 - x} \right) - \alpha x \frac{d^2}{dx^2} \left(\frac{1 - x^{q+1}}{1 - x} \right) \right\}$$

Differentiating and collecting terms

$$\sum_{t=1}^{q} F_t(\alpha, p) = \frac{1}{(p+1)} \{(p+1) - x^q[(p+1) + \alpha(pq - q^2)]\}$$

Since for all practical applications $p >> 1$, $p + 1 \sim p$. Then

$$\sum_{t=1}^{q} F_t(\alpha, p) = 1 - (1 - \alpha)^q \left[1 + \frac{\alpha}{p} (pq - q^2) \right] \tag{2}$$

Hence the weight integral distribution as a function of q can be calculated for various values of α from equation (2).

In order to analyze the results of the ultracentrifuge experiments, the molecular weight distribution expected from the random degradation of an initially monodisperse DNA solution has to be related to the corresponding distribution of sedimentation coefficients ($s_{20,w}$). Doty, McGill, and Rice (4) deduced an empirical relationship between $s_{20,w}$ and molecular weight for samples of DNA with molecular weights up to 10^7. This formula was obtained for sedimentation experiments conducted in a sodium chloride–citrate mixture (SSC). T7 DNA could not be satisfactorily studied in SSC or similarly dilute salt solutions since the values of $s_{20,w}$ were substantially less reproducible (± 8 per cent) than similar values measured in M sodium chloride (± 2 per cent). In the absence of definitive experiments, it has been tentatively assumed that the irreproducibility in low salt concentration results

from an instability of the sedimenting boundary at these low DNA concentrations. In the presence of a density gradient provided by the distribution of more concentrated salts in the centrifugal field, the boundary shape is more stable and the velocity reproducible. Since the $s_{20,w}$ of other DNA preparations is unchanged when the salt concentration of the solutions is varied from 0.05 to 1 M, it was concluded that the $s_{20,w}$ values obtained for T7 DNA in M NaCl could be directly compared with those measured in SSC. Moreover, an extrapolation of the formula to the T7 molecular weight (1.9×10^7) is presumably valid, since, as discussed elsewhere (2), the $s_{20,w}$ of T7 DNA is close to that predicted by the formula. Several Montroll-Simha theoretical integral distribution curves for various values of α, and in terms of $s_{20,w}$, are drawn in Fig. 1. It should be noted that these are smooth, upward-concave

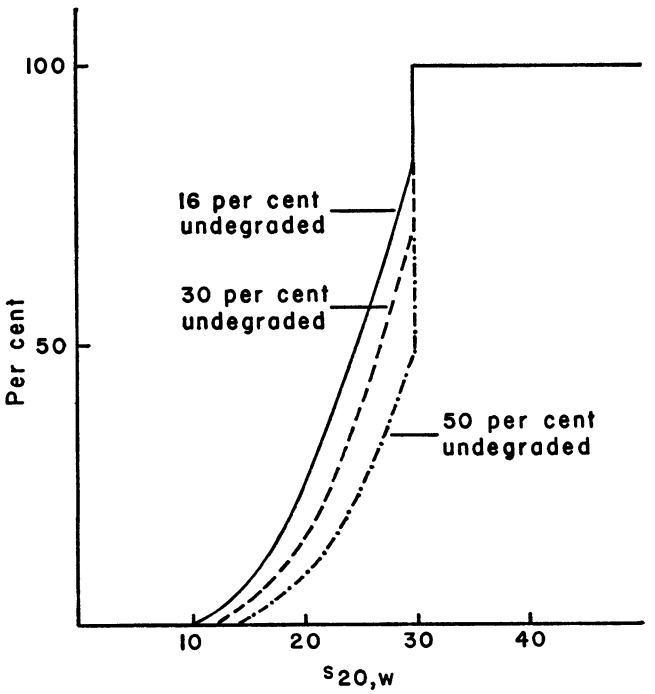

FIGURE 1 Theoretical integral distribution curves for three different degrees of random degradation for an ideal solution of DNA of $s_{20,w} = 30$ calculated from the Montroll-Simha theory and the relation between $s_{20,w}$ and molecular weight, according to Doty, McGill, and Rice (4).

curves. In contrast, a non-random process which, for example, splits molecules preferentially in half would result in a bimodal or more complex curve.

RESULTS AND DISCUSSION

The integral sedimentation diagrams of intact T7 DNA and of samples degraded

by x-rays, DNAase, shearing, and sonication are shown in Figs. 2–7. In each figure are also shown "calculated" curves obtained from equation (2) with a value of α corresponding to the experimentally determined fraction of molecules remaining undegraded in each experiment.

Ideally one would expect to obtain a step function for the integral distribution of a homogeneous DNA. The experimental distribution obtained is shown in Fig. 2. As discussed elsewhere (2), the lack of sharpness can probably be accounted for by

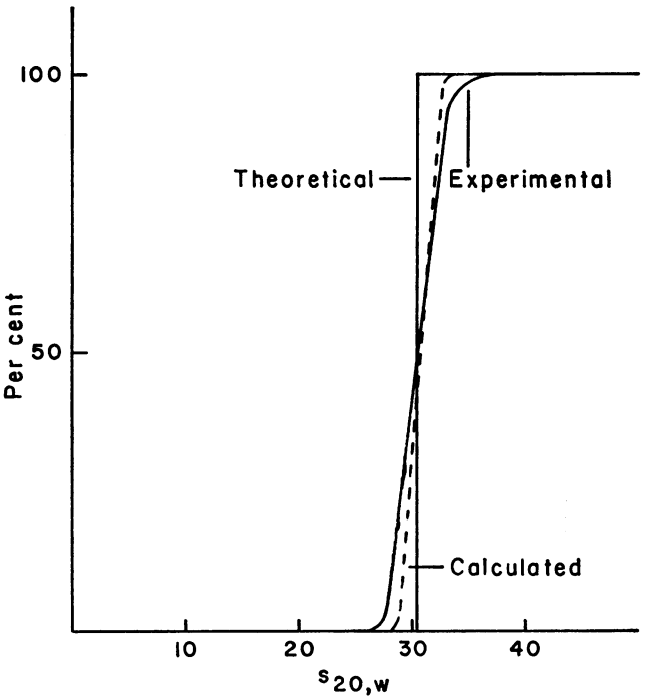

FIGURE 2 Integral distribution curve for T7 DNA. The "theoretical" curve shows the ideal distribution of a monodisperse solute. The "calculated" curve shows how this ideal distribution would appear experimentally; the distortion results from imperfections in the optical system of the ultracentrifuge and the finite width of the densitometer scanning slit (see reference 2). The "experimental" curve shows the sedimentation distribution of T7 DNA. The curvature at the upper end of this curve is the boundary anomaly referred to in the text.

(*a*) diffusion, (*b*) a boundary anomaly (resulting in curvature at the top of the trace), (*c*) optical imperfections in the ultracentrifuge, and (*d*) inability of the recording microdensitometer to represent accurately a razor edge (resulting in a slight broadening of the boundary due to the finite width of the scanning slit). These factors make a precise comparison between the theoretical and experimental curves difficult; however, the conclusions concerning the mode of degradation can be drawn from the qualitative differences between the distribution diagrams. The boundary

anomaly appears in the upper part of the densitometer trace of the intact DNA while the foot of the trace or the take-off point (if shearing has been avoided) is usually sharp and precise. Degradation results in the appearance of DNA of lower $s_{20,w}$, and the distribution of this DNA is of decisive importance whereas the upper part of the trace refers only to the undegraded DNA. The calculated curves are drawn as if this upper curvature did not exist; they have also been corrected approximately for optical imperfections in the ultracentrifuge and for the finite width of the densitometer scanning slit. The concentration dependence of $s_{20,w}$ was neglected; if this correction were known well enough to be applied, it would slightly cant the calculated curve but would not invalidate the qualitative comparisons from which our conclusions are derived.

Degradation of DNA by both x-ray irradiation and DNAase is generally assumed to occur through random scissions of each polynucleotide strand. Figs. 3 and 4 show that the sedimentation distribution diagrams of DNA degraded by these procedures approximate the shapes predicted from the Montroll-Simha theory. Some of this agreement may be fortuitous, since the diagrams may not be strictly comparable; if the single chain breaks introduce any flexibility into the normally rigid DNA mole-

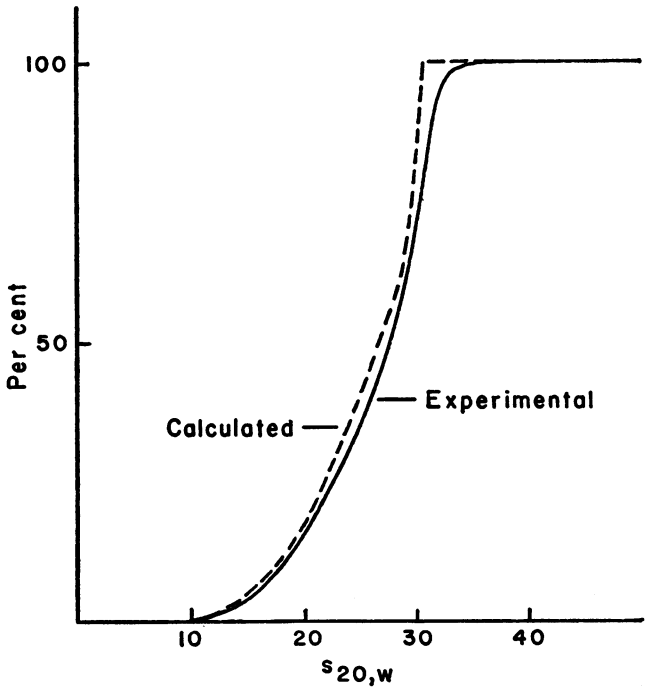

FIGURE 3 Integral distribution curve for T7 DNA degraded by x-rays (7680 roentgens). The calculated curve is for 40 per cent of the molecules undegraded; the inclination of the undegraded portion of the curve again reflects the corrections referred to in the legend of Fig. 2.

cule, the $s_{20,w}$-molecular weight relationship for these molecules may not conform to the relationship for undegraded DNA. However, the fact that the x-ray curve conforms closely to the theoretical curve gives tentative confirmation that the attack is random. The deviation of the DNAase curve from the Montroll-Simha distribution at low $s_{20,w}$ values might imply some degree of non-random attack, but further evidence would be required to substantiate this observation.

It should be noted that these x-ray and DNAase experiments do not imply that all bonds are equally susceptible to breakage since, if the susceptible bonds are randomly distributed, there would still be agreement with the predicted distribution. Furthermore, within the precision of the experiment small departures from randomness would not be observed.

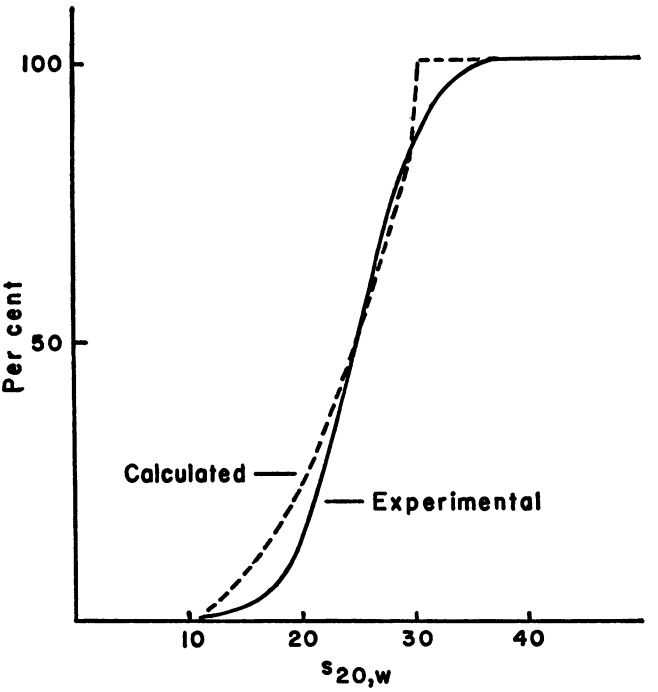

FIGURE 4 Integral distribution of T7 DNA partially degraded by DNAase. The curve is calculated for 20 per cent of the molecules undegraded.

Fig. 5 shows the sedimentation distribution diagram of T7 DNA after the solution had been passed three times at high pressure through a No. 27 hypodermic needle. Calculation shows that under the experimental conditions the flow through the needle was probably turbulent (Reynolds' number exceeded 3000) and hence the maximum shear gradient is unknown, though clearly sufficient to rupture a fraction of the DNA molecules. Shear degradation of polymers is expected to produce scission preferentially about the center of the molecules (6, 7), and therefore

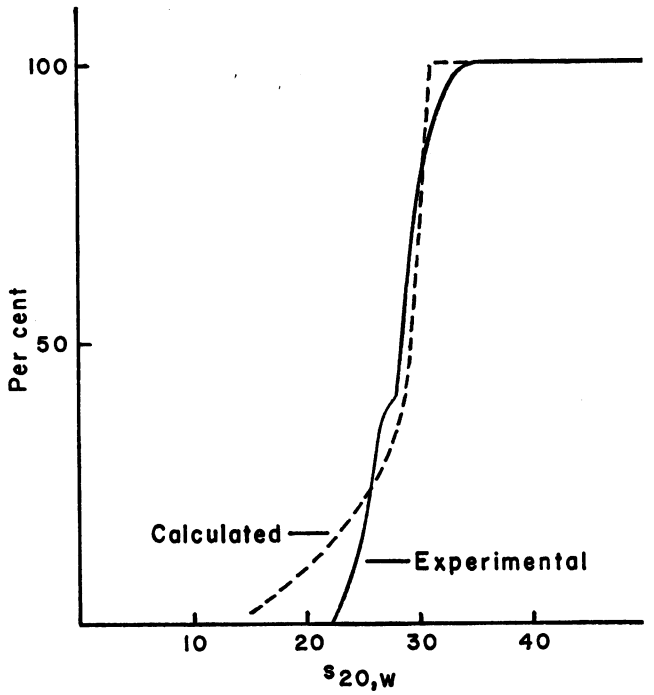

FIGURE 5 Integral distribution curve for T7 DNA sheared by three passages through a No. 27 hypodermic needle. The calculated curve is for 60 per cent of the molecules undegraded.

one would expect a bimodal distribution of sedimenting molecules in the sheared solutions; Fig. 5 confirms this expected bimodality.

Figs. 6 and 7 show the sedimentation distribution diagrams resulting from two different periods of sonication. A bimodal distribution similar to that produced by shearing is apparent, although the distribution of material in the lower $s_{20,w}$ peak is broader than in the case of shearing. This broadening is to be expected when the mechanism of sonic degradation is considered.

Sonic degradation of polymers does not occur in the absence of cavitation in the liquid (9, 10). Degradation probably occurs through the subjection of long-chain molecules to mechanical forces imparted by solvent flow which results from the cavitation (5). Calculations suggest that in the neighborhood of a collapsing cavity, solvent flow through the solute molecules produces a shearing stress which decreases with increasing distance from the cavity. At any moment solute molecules in a relatively large volume of solution are subject to weak stresses, while higher and higher stresses are imparted to molecules in progressively smaller volumes of the solution approaching the collapsing cavities. In an initially monodisperse DNA solution, a fraction of the molecules will meet stresses capable of degrading them

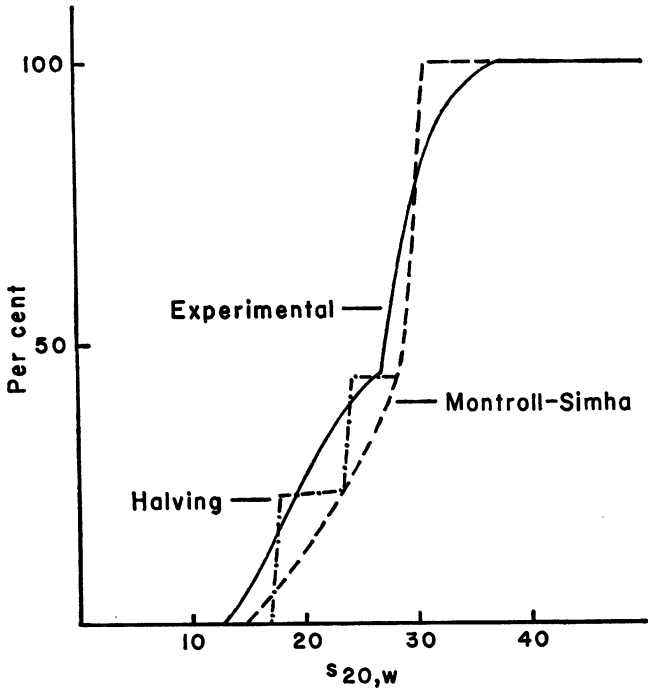

FIGURE 6 Integral distribution curve $s_{20,w}$ for DNA sonicated for 8 seconds. The Montroll-Simha curve is for 55 per cent of the molecules undegraded. The halving curve shows the expected distribution for an arbitrarily assumed mixture of 50 per cent intact, 25 per cent half, and 25 per cent quarter molecules.

to halves, while much smaller fractions will be quartered and further subdivided. Moreover, since the short duration of the shear stress probably does not permit the full extension of the molecule, the point of maximal shear stress will not lie at the center of the linear molecule but will be distributed about it at the instantaneous center of mass. This effect will broaden the distribution of molecular weights among the degraded molecules and obscure the resolution of quartered molecules and smaller fragments. One would therefore expect a distribution of degraded molecules appearing essentially bimodal, but skew on the side of lower $s_{20,w}$.

The kinetics of the sonic degradation also accords with the mechanism suggested above; the time needed to reduce a given population of molecules to fragments varies greatly according to the final median $s_{20,w}$ required (Fig. 8). Moreover, prolonged sonication at a given field strength will not take the $s_{20,w}$ below a certain value, which implies that shearing forces at a given field strength are incapable of breaking a molecule less than a critical size; to reduce a population of molecules to this size requires very long irradiation since the maximal stress is restricted to a very small volume of the solution in the sonic field.

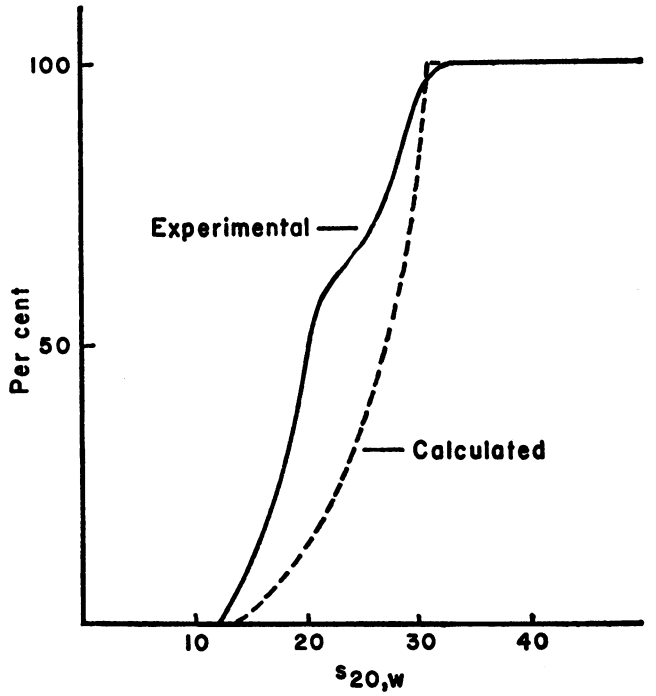

FIGURE 7 Integral distribution curve for T7 DNA sonicated for 10 seconds. The calculated curve is for 28 per cent of the molecules undegraded.

It has been suggested that some degradation in a sonic field results from attack by free radicals produced by cavitation. Such degradation would be expected to be random. If this process occurs in addition to degradation by mechanical forces, the molecular weight distribution would be expected to be further broadened, but at the low sonic intensities employed in these experiments it appears unlikely that free radical attack contributes significantly to the degradation since no protective effect from nitrogen, cysteine, or AET (S,2-aminoethyl isothiouronium bromide hydrobromide) in the solution could be demonstrated.

We conclude that x-rays, and DNAase at least in part, degrade DNA by a mechanism which, within the resolution of these experiments, appears to be randomly directed. On the other hand, sonication proceeds by means of shearing which results preferentially in the successive halving of the polymer. This conclusion was foreshadowed by the experiments of Oster (11) and Hodge and Schmitt (12) which demonstrated the halving and quartering of tobacco mosaic virus and tropocollagen, respectively, in a sonic field. Given sufficient time this process of successive halving will proceed until all the solute molecules have experienced the maximum shear forces engendered by the sonic field. In these circumstances the molecular weight of the polymer should range from the critical

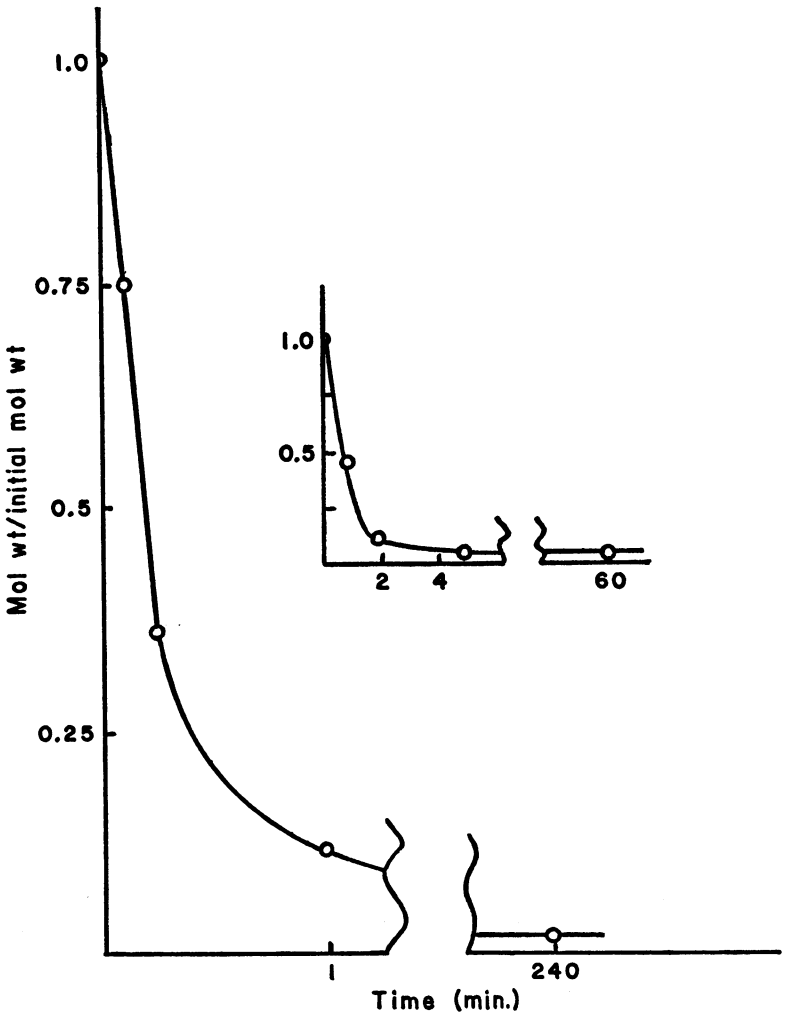

FIGURE 8 Decrease in molecular weight of T7 DNA with time of sonication. Insert: Similar curve for calf thymus DNA plotted from Table I of reference 4.

length capable of withstanding the maximum shearing stresses down to about half this length, *i.e.*, half the length of the molecules which the maximum stress was just sufficient to break.

Thus from long periods of sonication a molecular weight distribution very different from that obtained at intermediate periods would be predicted. This result has some bearing on the derivation of the formula which was utilized in calculating the results presented here. In the paper by Doty, *et al.* (4), the authors measured the median sedimentation coefficients and viscosities of polydisperse DNA samples. In order to obtain a linear extrapolation of the light-scattering data to zero angle,

they assumed that the molecular weight distribution in their sample was such that the weight average molecular weight was twice the number average (*i.e.*, a most probable distribution). It was further assumed that the sonicated samples each had a molecular weight distribution similar to the original. The authors justified their assumption by quoting electron microscope observations which suggested that sonic degradation resulted in random scission of the DNA molecules (13). This idea is incompatible with the results of the present paper. However, if, as has been suggested (7, 14), DNA isolated from any but viral sources represents the mechanically ruptured fragments of one or more initially very long chains, then the starting sample of calf thymus DNA used by Doty, *et al.*, probably had a molecular weight distribution essentially similar to those produced by sonication. It is therefore likely that the physical properties of samples of similar molecular weight distributions were compared; furthermore the error introduced by extrapolating the light-scattering data linearly was probably small (15).

The only sample which might have had a molecular weight distribution differing from the others was that produced by prolonged sonication. If the molecules in this fraction were limited to the twofold range of molecular weight predicted above, then the lowest calibration points obtained in the experiments of Doty, *et al.*, refer to a sample whose average properties are not strictly comparable to those of the remaining samples. However, this factor would be unlikely to displace significantly the lowest calibration point. It may therefore be concluded that, as an approximate correlation between the molecular weight, sedimentation coefficient, and viscosity of DNA, the relationship derived by Doty, *et al.*, remains valid.

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