At present, the evidence in support of a probabilistic control mechanism is based entirely on the results of one type of experiment, and alternative experimental approaches will be required in order to test the hypothesis. This work is under way. If the probabilistic view is found to be correct, it will still be necessary to determine what kind of control mechanisms could act to alter the probabilities and to define their modes of action.

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## *CLARIFICATION OF NATIVE DNA SOLUTIONS BY FILTRATION**

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The elimination of dust from solutions of nucleic acids has been a major barrier in obtaining reliable low-angle light-scattering data, which are essential for determining accurate molecular weights.[1] Most workers have relied upon extensive centrifugation with extremely careful handling of the resulting solutions. This procedure is time-consuming, and the results are not entirely satisfactory. Recently, Froelich, Strazielle, Bernardi, and Benoit[2] have recommended shaking the

DNA solutions with a 5 : 1 chloroform-isoamyl alcohol mixture and then centrifuging the aqueous layer of the emulsion at high speed; this technique, used with appropriate low-angle equipment, made it possible to obtain reproducible data at angles as low as 16°. In this paper we describe a method of clarification of native DNA solutions by filtration which is faster and more effective than previously described techniques and appears to have no damaging effects on the DNA preparations examined.

Filtration of solvents and of shear-insensitive solutions of random-coil polymers has long been used for the removal of dust prior to making light-scattering measurements. We have successfully used cellulose ester Millipore membranes for this purpose with both aqueous and organic solvents. Clarification of DNA solutions by filtering has seemed to present two serious problems: (*a*) absorption of the large nucleic acid molecules by the filter, and (*b*) breakage of the molecules by the hydrodynamic shear stresses encountered. Recently, Nygaard and Hall[3] have reported experiments which showed that 50–90 per cent of native T4 DNA passed through nitrocellulose membrane filters without clogging, and their results indicated that the DNA which passed through the filter was not denatured. Nygaard and Hall's data and estimates of the shear sensitivities of other viral nucleic acids suggested the following exploratory calculations and experiments on the possibility of filtering DNA solutions to remove dust.

*Estimates of Critical Shear Rates.*—The sensitivity of phage DNA to hydrodynamic shear and the resulting degradation of the molecules have been discussed by Hershey and Burgi[4] and by Levinthal and Davison.[5] In their studies on phage T2 DNA at concentrations less than 0.4 $\mu$g/ml, Levinthal and Davison established the existence of a critical shear rate (CSR), the highest nonturbulent flow rate through a given capillary below which there is no detectable molecular breakage. The hydrodynamic equation giving the maximum rate of shear at a capillary wall, $S_c$, for nonturbulent flow[6, 7] is

$$S_c = \frac{4F}{\pi r^3}, \tag{1}$$

where $F$ is the total flow of solution in cm$^3$/sec, and $r$ is the internal radius of the capillary. From equation (1) and the data of Levinthal and Davison, the CSR of T2 DNA (MW $\approx 12 \times 10^7$) was calculated to be $3.0 \times 10^4$ sec$^{-1}$. Davison and Freifelder[8] reported the CSR for halving T7 DNA (MW $\approx 2 \times 10^7$) to be approximately 50 times that for T2; thus, the estimated CSR of T7 is $1.5 \times 10^6$ sec$^{-1}$.

These estimates of critical shear rates for T2 and T7 DNA may be compared with the maximum calculable shear rates encountered during flow through the small-pore filters useful in light-scattering studies. For the commercially available equipment in use it was appropriate to assume the following: (*a*) a cellulose ester membrane with an average pore diameter of 0.22 $\mu$,[9] (*b*) a total pore area[9] of 10.2 cm$^2$, (*c*) a maximum total flow of solution through the filter, $F$, of 0.3 cm$^3$/sec, and (*d*) a flow of solution that could be described essentially as nonturbulent flow through a series of capillaries.[9] On the basis of these assumptions the maximum shear rate, $S_F$, at the wall of a capillary-like pore in the filter is given by

$$S_F = \frac{4F}{Ar}, \tag{2}$$

where $A$ is the total pore area and $r$ is the average radius of the pores. Filtering a solution of T2 DNA under the above conditions would subject the molecules to a maximum shear rate ($S_F$) of $1.1 \times 10^4$ sec$^{-1}$ which is only one third the CSR reported for T2. In addition, Hershey and Burgi[4] reported that the shear sensitivity of DNA solutions was less for higher concentrations, such as those used in this work, and this finding was confirmed by the capillary experiments of Levinthal and Davison.[5]

These considerations indicate the feasibility of forcing solutions of DNA as large as T2 through small-pore filters at flow rates low enough to avoid molecular shear. Consequently, we have undertaken an investigation of the effects of microfiltration on the concentration and on the intrinsic viscosity, $[\eta]$, which is quite sensitive to degradation of DNA molecules by shear.[10, 11]

*Experimental.*—Solutions of bacteriophage T7 DNA were prepared as described by Davison and Freifelder,[8, 12] and samples of bacteriophage T2 DNA were obtained by the method of Mandell and Hershey.[13] All measurements were made on solutions of DNA in the BPES phosphate buffer, pH 6.8, recommended by Crothers;[14] the composition of BPES is 0.006 $M$ $Na_2HPO_4$, 0.002 $M$ $NaH_2PO_4$, 0.001 $M$ disodium ethylenediaminetetraacetate, and 0.179 $M$ NaCl. (The sodium ion concentration of BPES is equivalent to that of the widely used SSC buffer;[15] however, BPES has the advantage of inhibiting bacterial growth, and solutions of DNA in this buffer are stable for several months.)

Concentrations were determined by optical absorbance measurements at 260 m$\mu$ on samples gravimetrically diluted with BPES to give absorbances of the order of 0.2. The extinction coefficients used for the samples in BPES were 0.0181 cm$^2$/$\mu$g T2 DNA[16] and 0.0198 cm$^2$/$\mu$g T7 DNA (estimated on the basis of composition). Data were collected on several T7 DNA solutions having concentrations between 50 and 500 $\mu$g/ml and on a T2 DNA solution of approximately 20 $\mu$g/ml.

The solutions were filtered through either a 0.22 $\mu$ (type GS) or a 0.45 $\mu$ (type HA) pore diameter cellulose ester Millipore membrane (47 mm diameter) supported in a stainless steel pressure holder. A pressure vessel[9] containing air at a desired pressure provided variable flow rates. The following technique was used for filtering all solutions. The dry filter holder was assembled with the appropriate filter membrane and rinsed by forcing approximately 50 ml of BPES through the membrane. The excess BPES was then shaken out of the holder. The DNA solution (up to 40 ml) was poured into the wet apparatus, and approximately one half of the sample was filtered only under the pressure of the head of liquid. This required about an hour. The pressure vessel was then connected, and in 1–2 min the remaining solution was collected separately after filtration at a higher pressure. The pressure used in all experiments maintained a flow rate such that the calculated shear rate in the filter did not exceed the previously mentioned critical shear rates for dilute T2 and T7 DNA solutions.

Viscosities, $\eta_r$, of the various samples relative to BPES were determined at 25°C in the low-shear rotating-cylinder viscometer described by Zimm and Crothers.[17] Intrinsic viscosities, $[\eta]$, in dl/gm have been approximated[18] by the quantity $(\ln \eta_r)/c$, which is nearly independent of concentration for both T2 and T7 DNA in BPES. To facilitate the comparison of results, viscosity measurements were made on solutions gravimetrically diluted, where necessary, to approximately the same convenient concentration (50 $\mu$g/ml for the T7 samples).

*Results and Discussion.*—Table 1 summarizes the data. Since the values of $(\ln \eta_r)/c$ were unaffected by filtration within the experimental error of the viscosity ($\pm 0.2\%$) and concentration ($\pm 1\%$) measurements, denaturation and molecular breakage are apparently insignificant.

The concentration data on unfiltered solutions and filtrates might be interpreted as evidence for partial ultrafiltration of the DNA. In all cases the concentration of the first fraction, which was filtered at low pressure, was less than that of the unfiltered material. In addition, the 112 $\mu$g/ml sample of T7 DNA filtered through the 0.22$\mu$ membrane, as well as the T2 DNA sample, showed an increase in the con-

TABLE 1

CONCENTRATION AND VISCOSITY DATA FOR FILTERED AND UNFILTERED SAMPLES OF T7 AND T2 DNA IN BPES

| Sample | Filter pore diam. ($\mu$) | Pressure for filtration (mm Hg) | Concentration of unfiltered solution or of filtrate ($\mu$g/ml) | Concentration of solution for viscosity measurements (gm $\times$ $10^3$/dl) | $\eta_r$ | $\frac{\ln \eta_r}{c} \approx [\eta]$ (dl/gm) |
|---|---|---|---|---|---|---|
| T7 DNA | 0.45 | (Unfiltered) | 61.0 | 6.10 | 1.884 | 104 |
| " | " | ~5 | 57.3 | 5.73 | 1.817 | 104 |
| " | " | 130 | 59.2 | 5.92 | 1.859 | 105 |
| " | " | (Unfiltered) | 120 | 6.04 | 1.839 | 101 |
| " | " | ~5 | 106 | 5.33 | 1.755 | 106 |
| " | " | 130 | 113 | 5.68 | 1.813 | 105 |
| " | " | (Unfiltered) | 585 | 5.81 | 1.823 | 103 |
| " | " | 130 | 482 | 4.82 | 1.654 | 104 |
| " | 0.22 | (Unfiltered) | 58.7 | 5.87 | 1.842 | 104 |
| " | " | ~5 | 56.2 | 5.62 | 1.746 | 99 |
| " | " | 260 | 57.7 | 5.77 | 1.831 | 105 |
| " | " | (Unfiltered) | 112 | 5.71 | 1.825 | 105 |
| " | " | ~5 | 81 | 4.04 | 1.523 | 104 |
| " | " | 130 | 124 | 6.28 | 1.952 | 106 |
| " | " | (Unfiltered) | 519 | 5.02 | 1.704 | 106 |
| " | " | 130 | 383 | 3.86 | 1.520 | 108 |
| T2 DNA | 0.22 | (Unfiltered) | 23.8 | 2.38 | 2.038 | 299 |
| " | " | ~5 | 16.6 | 1.66 | 1.603 | 283 |
| " | " | 50 | 27.5 | 2.75 | 2.269 | 298 |

centration of the high-pressure filtrate over that of the unfiltered material. Although the decrease in concentration could be explained by dilution of the first fraction of the filtrate by residual solvent from the preceding rinse, the fact that both decreases and increases were observed is evidence for partial ultrafiltration; i.e., some molecules are prevented from passing through the filter at low pressure, but at high pressure the remaining molecules are pushed through the membrane, thus giving a high-pressure filtrate with a concentration greater than that of the unfiltered solution.

The concentration changes may also be due to some absorption of the DNA by the membrane, as reported by Nygaard and Hall.[3] At high DNA concentrations (greater than 500 $\mu$g/ml) our filtrates were less concentrated than the original unfiltered material, even when the filtration was carried out at a high pressure. Therefore, it might be advisable to avoid clarifying such concentrated solutions by this procedure.

The observations of Nygaard and Hall,[3] who used nitrocellulose membrane filters, suggest that denatured DNA might be absorbed by the Millipore cellulose ester filters. Preliminary experiments in our laboratory on filtration of both slow-cooled and fast-cooled samples of heat-denatured T7 DNA indicated that 50 per cent or more of the UV-absorbing material is removed from the filtrate, thus apparently eliminating this method for direct clarification of denatured material. Further work must be undertaken to fully characterize the effects on denatured DNA of filtration through the cellulose ester membranes.

Exploratory low-angle light-scattering measurements were carried out on filtered T7 DNA solutions to determine whether dust can be removed effectively. The

results are close to those which we obtained earlier on samples clarified by centrifugation. However, the stability of low-angle readings, which is difficult to attain by centrifugation techniques, is substantially improved by filtering. A thorough low-angle light-scattering study on DNA solutions clarified by filtration is now in progress.

*Summary.*—The desirability of using small-pore cellulose ester filters for easy and quick removal of dust particles from DNA solutions for light-scattering measurements has been indicated. Since the major obstacle to filtration was the shear sensitivity of polynucleotides, calculations based on the known shear sensitivities of T2 and T7 DNA have been presented to indicate the theoretical feasibility of this clarification technique. Viscosity data have demonstrated that, under the proper conditions, solutions of native DNA can be filtered without breaking the molecules. Moreover, preliminary low-angle light-scattering results have indicated that filtration is more effective than centrifugation in clarifying native DNA solutions. Filtration cannot be recommended at present for direct clarification of denatured DNA solutions, since denatured material appears to be partially retained by the filter.

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