Hopton and Linnett, to discuss energetic effects in terms of the more classical electrostatic interactions plus correlation effects.

We would conclude from consideration of the natural orbital analysis of $H_3^+$ that in the bonding region, linear $H_3^+$ has many similarities to $H_2$. In particular, the description of "nonpairing" offered by Hopton and Linnett is no different in kind from the electron correlation in $H_2$, and we believe it an unfortunate choice of terminology to refer to this as nonpaired. We would prefer to retain the concept of the one-electron bond for those cases in which there is a single electron in a bonding region which is *largely spin uncorrelated* with other electrons, and this situation does not apply for $H_3^+$. Because there are two effective bonding regions in linear $H_3^+$ and only two electrons, it follows that on the average there is only one electron in each region, but it does not follow that the electrons are paired in any sense different from that in the case of the hydrogen molecule.

In fact, every facet of our analysis suggests that there is not any difference in kind between linear $H_3^+$ and $H_2$. The former might therefore be better considered as a relatively normal (albeit lengthened) single bond in which is embedded an additional proton. Of course, it should be borne in mind that the present analysis is for the hypothetical (nonequilibrium) linear symmetric case using a relatively crude calculation. More accurate calculations are in progress, and we hope eventually to make a more definitive analysis. Even in the linear case, however, we can expect some significant changes. For example, the calculation of Hirschfelder, Eyring, and Rosen did not allow orbital exponents to vary. We can expect a significant increase in bond concentration toward the axis in $H_3^+$ as compared to $H_2$ at comparable distances, but the present crude calculations are not sufficiently refined to show this. We do expect, however, that this concentration will not have any significant effect upon the importance of electron correlation as discussed here.

# Dynamic Mechanical Properties of Poly-γ-benzyl-L-glutamate in a Helicogenic Solvent

By N. W. Tschoegl and John D. Ferry

The storage ($G'$) and loss ($G''$) shear moduli have been measured over a wide frequency range for dilute solutions of poly-γ-benzyl-L-glutamate in $m$-methoxyphenol, a helicogenic solvent, using the apparatus of Birnboim and Ferry. A few measurements were also obtained in dichloroacetic acid, which produces the random coil configuration. The temperature dependence of the viscoelastic properties was satisfactorily described by the method of reduced variables, from 0 to 50° in $m$-methoxyphenol (1 and 2% polymer), and at −6 and 0° in dichloroacetic acid (2% polymer). The frequency dependence of $G'$ and $G''$ in the $m$-methoxyphenol solutions was qualitatively intermediate between the predictions of the Kirkwood–Auer theory for rigid rods and the Zimm theory for flexible random coils with dominant hydrodynamic interaction. A fit to the Kirkwood–Auer theory at low frequencies gave values for the molecular weight and relaxation time which were not unreasonable considering that there must be substantial intermolecular interaction even at 1% concentration. A fit to the Zimm theory gave unacceptable values for the molecular weight and terminal relaxation time. Thus the hydrodynamic behavior revealed at low frequencies is essentially rodlike, but the deviations from the Kirkwood–Auer theory at high frequencies indicate some flexibility of the helix. In dichloroacetic acid, the data are of limited scope; the magnitude of the terminal relaxation time is much too small for rigid rods but is somewhat larger than that predicted by the Zimm theory for coils.

## Introduction

The characteristic hydrodynamic behavior of synthetic polypeptides in the helical form, associated with a stiff rodlike configuration, has been investigated by several types of measurements, including non-Newtonian viscosity[1] and electrical birefringence.[2,3] It should also be manifested in viscoelastic measurements. Kirkwood and Auer[4] showed over a decade ago that a solution of thin rodlike molecules should reveal viscoelastic properties when subjected to sinusoidal deformations, and they calculated the frequency dependence of both viscous and elastic components of the mechanical response in terms of molecular parameters.

We describe here some viscoelastic measurements of dilute solutions of poly-γ-benzyl-L-glutamate in a helicogenic solvent, and compare the results with the Kirkwood–Auer theory. A few data are also presented for the same polypeptide in a solvent which favors the random coil configuration.

## Theory

The viscoelastic properties of a dilute macromolecular solution are conveniently described by the contributions of the solute to the components of the complex shear modulus, $G^* = G' + iG''$. The storage modulus $G'$ is due entirely to the solute; the portion of the loss modulus contributed by the solute is $G'' - \omega v_1 \eta_s$, where $\omega$ is the circular frequency, $v_1$ the volume fraction of solvent (usually negligibly different from unity), and $\eta_s$ the solvent viscosity. According to the Kirkwood–Auer theory[4] for a rodlike macromolecule, these quantities are given by

$$G' = (3cRT/5M)\omega^2\tau^2/(1 + \omega^2\tau^2) \qquad (1)$$

$$G'' - \omega v_1 \eta_s = (3cRT/5M)\omega\tau[1/(1 + \omega^2\tau^2) + {}^1\!/_3] \qquad (2)$$

where $c$ is concentration of solute in g./cc., $M$ is the molecular weight, and $\tau$, the relaxation time, is given by

$$\tau = \pi\eta_s L^3/18kT \ln (L/b) \qquad (3)$$

Here $L$ is the length of the rod, and $b$ the length of the

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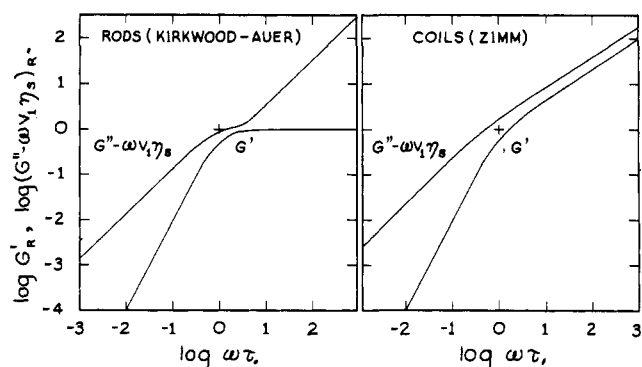(4) J. G. Kirkwood and P. L. Auer, *J. Chem. Phys.*, **19**, 281 (1951).

Fig. 1.—Logarithmic plots for the contributions of a macromolecular solute to the components of the complex shear modulus, as predicted by the theories of Kirkwood and Auer for rigid rods and Zimm for flexible coils.



Fig. 2.—Logarithmic plots of $G'$ (black circles) and $G'' - \omega v_1 \eta_s$ (open circles) for 1% poly-γ-benzyl-L-glutamate in $m$-methoxyphenol, reduced to 25°. Pip down, measured at $-0.1°$; left, 25.0°; up, 50.3°. Solid curves represent Kirkwood–Auer theory with coordinates adjusted so that the cross corresponds to origin of left side of Fig. 1. The subscript p denotes multiplication by $T_0 \rho_0/T \rho$.

repeating unit of which the rod is considered to be composed.

It is useful to define dimensionless variables as

$$G'_R = G'(5M/3cRT) \quad (4)$$

$$(G'' - \omega v_1 \eta_s)_R = (G'' - \omega v_1 \eta_s)(5M/3cRT) \quad (5)$$

so that curves of $G'_R$ and $(G'' - \omega v_1 \eta_s)_R$ against the dimensionless frequency $\omega\tau$ can depict the theory for any molecular weight or concentration. Such dimensionless plots are shown in Fig. 1 and are compared there with similar dimensionless plots for the viscoelastic properties of randomly coiled macromolecules in dilute solution predicted by the theory of Zimm,[5] which is appropriate to coils with dominant hydrodynamic interaction in a Θ-solvent.[6] (The normalizing factors are somewhat different for the Zimm theory; the factor $^3/_5$ does not enter in the modulus, and the dimensionless frequency is $\omega\tau_1$, where $\tau_1$ is the terminal or longest relaxation time.) The marked differences in shape at high frequencies distinguish clearly between the stiff rod and the flexible coil. For the rod, $G'$ becomes constant and $G'' - \omega v_1 \eta_s$ is directly proportional to $\omega$, reflecting a single relaxation time. For the coil, both quantities are proportional to $\omega^{2/3}$, reflecting a spectrum of relaxation times as specified by the Zimm theory. Of course, intermediate degrees of stiffness (broken rods, hinge points, and wormlike coils[7]) would be expected to manifest an intermediate type of behavior. Viscoelastic properties may be a more sensitive index of such partial flexibility than other hydrodynamic measurements which are dominated by the longest relaxation times.

### Experimental

**Materials.**—A sample of poly-γ-benzyl-L-glutamate was generously furnished by Professor M. A. Stahmann and Dr. Sudhakar Wagle of the Department of Biochemistry. It was believed to have a fairly sharp molecular weight distribution based on chromatographic analysis following conversion to the polyglutamic acid.[8] Its intrinsic viscosity at 25° in dichloroacetic acid was 1.83 dl./g., corresponding to a molecular weight of 345,000 according to the equation given by Doty, Bradbury, and Holtzer.[9]

As a helicogenic solvent with the rather high viscosity which is required for observation of viscoelasticity at relatively low fre-
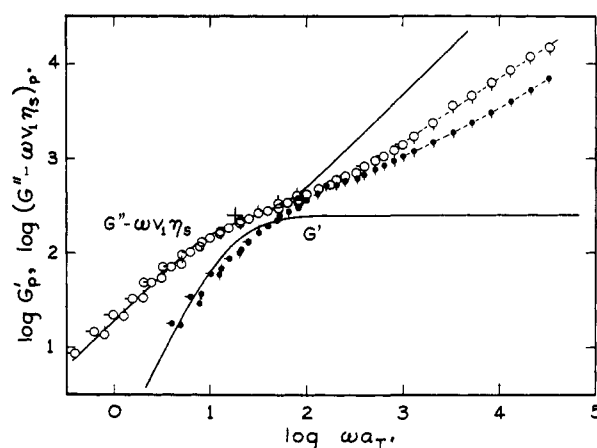
quencies,[10] $m$-methoxyphenol was chosen. The Eastman product was redistilled under vacuum. Its density was measured pycnometrically between the temperatures of 0 and 50° and could be represented by the equation $\rho = 1.1575 - 0.0006755t$, where $t$ is the centigrade temperature. Its viscosity was measured with a capillary viscosimeter over the same temperature range and could be represented by the equation

$$\log \eta = 1.119 - 4.19(t + 9.77)/(t + 66.9) \quad (6)$$

Solutions of the polymer in the methoxyphenol were mixed with a slow magnetic stirrer at temperatures not above 30°, and required about 3 days for complete dissolution. They were used without delay to avoid danger of oxidation of the solvent. To make sure that the polymer was in the helical form, measurements of rotatory dispersion were made at a concentration of 2% at 26° between 385 and 620 mμ in a Rudolph Model 200S spectropolarimeter.[11] The dispersion closely resembled that found for the same polymer in $m$-cresol by Yang and Doty[12] and identified with the helical form.

Dichloroacetic acid, in which the polymer assumes a random coil configuration, was used as a solvent for one solution. The Eastman product (practical grade) was redistilled. At $-5.7$ and 0.0°, the temperatures chosen for viscoelastic measurements in this solvent, the densities were 1.588 and 1.581 g./ml., respectively; the viscosities were 0.224 and 0.165 poise.

**Method.**—The storage and loss moduli, $G'$ and $G''$, of the solutions were measured over a frequency range from 0.016 to 400 cycles/sec. by the apparatus of Birnboim and Ferry[13] with modifications which have been described in subsequent publications.[10,14] The solution is sheared between a stationary cup and an immersed rod which oscillates along its axis with a very small excursion. For the $m$-methoxyphenol solutions, the rod and cup were of stainless steel as in previous studies of various polymers[10,14]; for the dichloroacetic solution, a rod of gold-plated aluminum and cup of gold-plated brass were employed.

### Results

Solutions in $m$-methoxyphenol containing 1 and 2% polymer by weight were studied at approximately 0, 25, and 50°; the exact temperatures are given in the legends of Fig. 2 and 3. The data are shown after reduction to a reference temperature of 25° by the usual method of reduced variables.[15] For this purpose,

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(11) We are much indebted to Mr. John W. Allis for making these measurements, and to Professor Robert A. Alberty for the use of the instrument.

(12) J. T. Yang and P. Doty, *J. Am. Chem. Soc.*, **79**, 761 (1957).

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(15) J. D. Ferry, "Viscoelastic Properties of Polymers," John Wiley and Sons, Inc., New York, N. Y., 1961, Chapter 11.
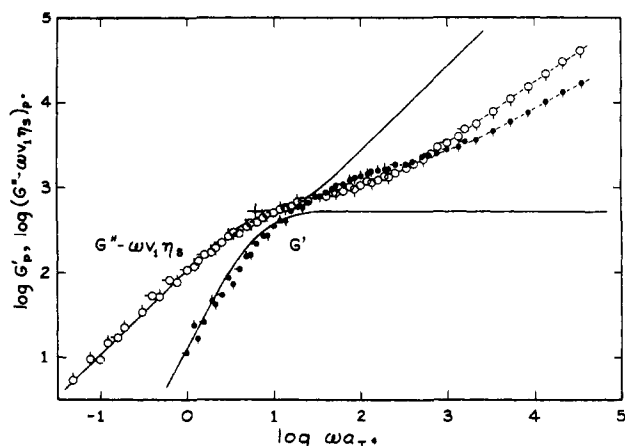
Fig. 3.—Logarithmic plots of $G'$ (black circles) and $G'' - \omega v_1 r_s$ (open circles) for 2% poly-$\gamma$-benzyl-L-glutamate in $m$-methoxyphenol, reduced to 25°. Pip down, measured at $-0.1°$; left, 25.0°; up, 49.9°. Solid curves represent Kirkwood–Auer theory with origin of dimensionless plot at location of cross.

$G'T_0\rho_0/T\rho$ and $(G'' - \omega v_1\eta_s)T_0\rho_0/T\rho$ are plotted logarithmically against $\omega a_T$. Here $\rho$ and $\rho_0$ are the solution densities at the absolute temperature of measurement $T$ and the reference temperature $T_0 = 298°K$. The shift factors $a_T$ were determined empirically for the best fit, rather than calculated from the contribution of polymer to steady flow viscosity,[10] because the latter could not be reliably determined at each temperature. However, as shown in Table I, the empirical values are quite close to, but slightly greater in magnitude than, those derived from temperature dependence of the solvent viscosity and can therefore be used with some confidence.

TABLE I

TEMPERATURE SHIFT FACTORS FOR SOLUTIONS IN $m$-METHOXYPHENOL (REFERENCE 25.0°)

| Concn., % | Temp., °C. | log $a_T$ temp. | log $a_T$ from $\eta_s$[a] |
|---|---|---|---|
| 1 | $-0.1$ | 1.11 | 1.01 |
|   | 50.3 | $-0.70$ | $-0.60$ |
| 2 | $-0.1$ | 1.13 | 1.01 |
|   | 49.9 | $-0.71$ | $-0.58$ |

[a] Calculated as log $\eta_s T_0\rho_0/\eta_{0s}T\rho$.

Plots of the reduced storage and loss moduli against $\omega a_T$ are shown for the 1 and 2% solutions in Fig. 2 and 3. The superposition of the points from the different temperatures is quite good, and it may be concluded that there is no significant change in molecular configuration or interaction over this temperature range, but merely a change in all the relaxation times approximately in proportion to the solvent viscosity. The shapes of the curves are intermediate between the two extreme theoretical types shown in Fig. 1.

At low frequencies, $G'$ is found to be proportional to $\omega^2$ and $G'' - \omega v_1\eta_s$ is proportional to $\omega$, with slopes of 2 and 1, respectively, on logarithmic scales. This behavior is expected for any linear viscoelastic system and in this region the data can be matched to either the Kirkwood–Auer or the Zimm theories. However, a Zimm match leads to unacceptable values of molecular parameters, as described below, and so only the Kirkwood–Auer match is drawn in Fig. 2 and 3, obtained by suitable horizontal and vertical shifts which place the origin of the theoretical dimensionless plots at the location indicated by a cross.
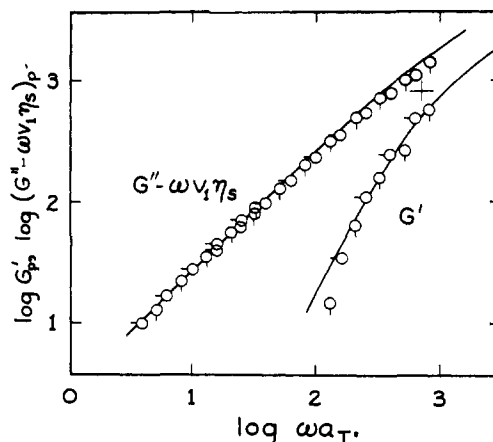


Fig. 4.—Logarithmic plots of $G'$ and $G'' - \omega v_1\eta_s$ for 2% poly-$\gamma$-benzyl-L-glutamate in dichloroacetic acid, reduced to 0°. Pip down, measured at $-5.7°$; left, 0.0°. Solid curves represent Zimm theory with origin of dimensionless plot at location of cross.

In accordance with eq. 4 and 5, the location of the cross on the ordinate scale is log $3cRT/5M$, from which the molecular weight can be calculated; on the abscissa scale it is $-\log \tau$. These values are given in Table II. Similarly, from the location of the origin in a Zimm match, the molecular weight and the terminal relaxation time of the spectrum, $\tau_1$, can be calculated as described in earlier papers on random coil polymers.[10,14] These are also given in the table.

TABLE II

PARAMETERS OBTAINED FROM KIRKWOOD–AUER AND ZIMM THEORIES FOR SOLUTIONS IN $m$-METHOXYPHENOL

| | | |
|---|---|---|
| Concn., weight % | 1.00 | 2.00 |
| Concn., g./ml. | 0.0114 | 0.0228 |
| Viscosity at 25°, poise | 21.2 | 107 |
| | | |
| Kirkwood–Auer match | | |
| Cross ordinate | 2.40 | 2.71 |
| log $M$ | 5.83 | 5.82 |
| log $\tau$ | $-1.26$ | $-0.79$ |
| log $\tau$ calcd. from eq. 3 with $\eta_s$ | $-2.05$ | $-2.05$ |
| log $\tau$ calcd. from eq. 3 with $\eta$ | $-0.25$ | 0.45 |
| | | |
| Zimm match | | |
| Cross ordinate | 1.95 | 2.36 |
| log $M$ | 6.50 | 6.39 |
| log $\tau_1$ | $-1.00$ | $-0.66$ |
| log $\tau_1$ calcd. from eq. 7 | $-3.98$ | $-3.98$ |

One solution with 2% polymer by weight in dichloroacetic acid was studied at $-5.7$ and $0.0°$. The data are shown in Fig. 4, reduced to 0°. They are somewhat sparse because of the difficulty of making measurements in a solvent of considerably lower viscosity than usual in such work, and they do not extend to high enough reduced frequencies to distinguish between the shapes of the Zimm and the Kirkwood–Auer dispersions. They are matched to the theoretical Zimm curves in the figure.

## Discussion

**Behavior in $m$-Methoxyphenol.**—Since log $M$ from intrinsic viscosity is 5.54, it is evident from Table II that application of the Kirkwood–Auer theory to the dynamic data gives apparent molecular weights which are too high by about a factor of two. On the other hand, the Zimm theory would provide apparent weights nearly tenfold too high, entirely unacceptable.

It may be remarked that if there is a distribution of molecular weights, a complicated average value is obtained from each of these matches to theory. It can be shown that for the Kirkwood theory the average molecular weight is $M_{z+3}M_{z+2}M_{z+1}/\overline{M_zM_w}$; for the Zimm theory it is $M_zM_wM_n^2/(\overline{M^{3/2}})^2$, where $\overline{M^{3/2}}$ is $\int M^{3/2}\varphi(M)\mathrm{d}M$, $\varphi(M)$ being the number distribution of molecular weights. Since the Kirkwood average is very heavily weighted by species of high molecular weight, a small degree of heterogeneity could easily cause the apparent molecular weight to be too high by a factor of two, as observed. But the Zimm average is only moderately weighted on the high side, and it would take an extremely broad distribution to give a tenfold discrepancy. Thus the data at low frequencies conform much better to the Kirkwood–Auer theory.

A similar conclusion is reached by comparing the relaxation times. To calculate theoretical Kirkwood–Auer relaxation times from eq. 3, it is necessary to assign values to $L$ and $b$. Although the helix length per amino acid residue is usually taken to be 1.5 Å., recent evidence[3,16] suggests an alternative of 2.0 Å. If the latter is used, $L = 3150$ Å. The assignment of $b$, the length of a hydrodynamic unit, is uncertain, but the final result is insensitive to the choice; we take it as 20 Å. If $\eta_s$ in eq. 3 is assumed to represent the solvent viscosity, $\log \tau$ is calculated to be $-2.05$, which is somewhat below the experimental values. A difference in this direction is to be expected since the theory holds only for infinite dilution and there must be substantial hydrodynamic interference of the rods at 1 or 2% concentration. If the solution viscosity $\eta$ is substituted for $\eta_s$, an over-correction would be expected, and the corresponding values in Table II show that this is the case. If the length per residue is taken as 1.5 instead of 2 Å., the experimental values of $\tau$ still lie between those calculated from $\eta_s$ and from $\eta$. Thus application of the Kirkwood theory leads to reasonable results for $\tau$. Alternatively, the Zimm match can be used to calculate $\tau_1$ from the equation for coils[5,10]

$$\tau_1 = 0.200\eta_z a^3 Z^{3/2}/kT \qquad (7)$$

where $\eta_z$ is the local viscosity, again taken as that of the solvent, $Z$ is the degree of polymerization, and $a^2$ the mean square length per monomer unit. If a reasonable estimate of 10 Å. is made for $a$, the calculated values of $\tau_1$ are too small by a factor of $10^3$, showing that the coil model is not at all applicable.

Nevertheless, the rigid rod theory does not describe the viscoelastic results at higher frequencies, where $G'$ increases with about the 0.55 power of frequency instead of approaching an asymptotic value. This behavior probably reflects a moderate flexibility of the helix, which has also been inferred from other measurements.[16,17] Unfortunately, no theory is yet available to gage the flexibility quantitatively from these results.

From the curves of $G'$ and $G'' - \omega v_1\eta_s$, it is possible to calculate a distribution function of relaxation times

by conventional approximation methods; separate calculations from the two components give results in fairly good agreement. In a formal manner, this distribution could be attributed solely to a distribution of rod lengths if the rods were all completely rigid. The corresponding distribution of molecular weights $\varphi(M)$ turns out to be meaningless, however, since its integral is severalfold greater than unity. Moreover, a theoretical calculation of $G'_R$ and $(G'' - \omega v_1\eta_s)_R$ for a most probable distribution of molecular weights (evaluated with the generous cooperation of Professor S. E. Lovell of the Theoretical Chemistry Institute) gives curves entirely different in shape from those in Fig. 2 and 3; that for $(G'' - \omega v_1\eta_s)_R$ differs from the corresponding curve for uniform molecular weight only in the region of the inflection, which is more gradual, extending over about two decades instead of one. Thus, the distribution of relaxation times cannot be explained by molecular weight distribution, and to ascribe it to moderate flexibility seems most plausible.

In the 2% solution (Fig. 3), the curve for $G'$ crosses that for $G'' - \omega v_1\eta_s$ twice, so the storage component exceeds the loss in the intermediate frequency range. Such behavior is not predicted by any dilute solution theory, nor has it been observed in extensive studies of flexible coil polymers such as polystyrene[10,18] and polyisobutylene[14,19] including molecular weights up to 1,700,000 at 2% concentration. However, it is often observed in undiluted polymers, and it is undoubtedly associated here with intermolecular interference at the higher concentration. There is evidence of such interference in the dynamic mechanical properties of desoxyribonucleic acid[20] at a concentration of 1.2 g./dl.

**Behavior in Dichloroacetic Acid.**—Similar matches of the data of Fig. 4 to the theories of Kirkwood and of Zimm (cross as drawn) give apparent values for $\log M$ of 5.37 and 5.93, respectively. The former is too low and the latter too high. In view of the usual pattern,[10,14] the latter is more realistic. The Kirkwood theory gives the calculated values of $\log \tau$ as $-2.33$ from $\eta'$ and $-1.16$ from $\eta$; the apparent value is $-2.98$ which, instead of lying between them, is smaller than either. The Zimm theory gives a calculated value of $\log \tau_1$ as $-4.26$ compared with an observed value of $-2.85$. It may be concluded that the data in dichloroacetic acid are definitely inconsistent with rigid rods, as expected, but do not agree very well with the Zimm theory for coils either. Attempts to find coil-favoring solvents of higher viscosity, in which better measurements could be obtained, have not yet been successful.

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