Our observation of enhanced T_4 deiodination by phagocytosing human leukocytes is evidence that the deiodination reaction in human tissue is mediated by a peroxidase–hydrogen peroxide system, in agreement with previous observations in several tissues of the rat (*3*). In addition, our observations indicate that T_4 can serve as a source of iodine for iodination reactions within the leukocyte, and might, in this way, fulfill a possible microbicidal function.

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Polymers: Synthesis and Characterization of Extremely High-Molecular-Weight Polystyrene

Abstract. *Polystyrenes with molecular weights up to 44×10^6 grams per mole have been characterized by light-scattering and equilibrium ultracentrifugation methods. The Mark-Houwink equation, which relates the molecular weight and the intrinsic viscosity of flexible polymers, can be used only if the measurements are made in a theta solvent at the theta temperature.*

Among polymer scientists there has been an unspoken understanding that for synthetic polymers, unlike naturally occurring biopolymers, there has heretofore been a real limitation in the attainment of extremely high molecular weights. We know of no systematic attempts to examine polymers with molecular weights greater than 9×10^6 g mole^{-1}. This report demonstrates that "monodisperse" polystyrenes with molecular weights up to 44×10^6 can be synthesized and characterized. The highest molecular weight we report does not signify a limit in terms of chemical synthesis, but it does represent a value beyond which methods for the characterization of molecular weight become more difficult.

Many biopolymers have extremely high molecular weights. The molecular weight of a linear bacteriophage DNA is reported (*1*) to be 157×10^6 g mole^{-1}. A simple enzymatically produced homopolymer that has molecular weights up to 500×10^6 is dextran, either branched or linear (*2*). However, such molecules have broad molecular weight distributions and are exceedingly difficult to fractionate well. It would be of interest to the biological chemist to have large molecules that exhibit some of the same viscoelastic, shear-degradative, and conformational properties as the naturally occurring polymers.

For the polymer chemist the availability of one or more decades of molecular weight allows an accurate test of theoretical concepts of polymer thermodynamics, conformation, and hydrodynamics. For example, radii of gyration can easily be measured over two decades of molecular weight so that asymptotic expansions of theoretical equations relating conformation and excluded volume can be compared with experimental data.

We synthesized the high-molecular-weight polystyrene samples by using high-vacuum techniques and purification procedures described elsewhere (*3*). The solvent used was tetrahydrofuran (THF), and the "initiator" was low-molecular-weight polystyryllithium in benzene.

The polymerization of styrene with an organolithium initiator in either a hydrocarbon or ether solvent leads to the synthesis of an atactic (*4*) polymer of narrow molecular weight distributions (*5*). Samples 13 and 18 prepared in the work reported here were shown by gel permeation chromatography (GPC) to have reasonably narrow molecular weight distributions with no indication that either sample possessed a "low" molecular weight fraction. The recognized modes of spontaneous termination of polystyryllithium in THF involve reaction with solvent (*6*) and a possible isomerization reaction (*7*). There is no known termination or transfer step (metallation) that can lead to the synthesis of branched polystyrene or microgel in the anionic polymerization of styrene.

After initiation, the polymerization flask was maintained for several hours at 25°C. Then the flask was broken open and the contents were placed in a 3-liter beaker containing benzene. The jelly-like mass from the reaction flask was repeatedly soaked in fresh benzene. Over the course of 3 weeks 5 gallons (18.9 liters) of benzene were used to partition out the last traces of THF. The remaining loosely held gel (having the consistency of gelatin) was then freeze dried from its benzene solution. In this manner 80 percent of the original 20 g was recovered. The freeze-dried polystyrene was then used to make solutions for all subsequent measurements.

The dissolution process is so slow that 2 to 3 weeks were required to make solutions, just as the initial partitioning of THF with benzene through a polymer gel was possible only because of the slow dissolution and disentanglement. The very dilute solutions used in this work ($\sim 10^{-5}$ g/ml) had a faint suggestion of stringiness. We noticed very early in the preparation procedure that the viscoelasticity of the solutions would decrease upon continued stirring. Subsequently, all solutions were shaken or stirred very carefully for equivalent times. These effects are similar to those reported for nucleic acid solutions.

The initial experiments on other

Table 1. Molecular constants of two high-molecular-weight polystyrenes.

Sample No.	Solvent	Temperature (°C)	$M_w \times 10^{-6}$ (g mole^{-1})	$\langle S^2 \rangle_z^{1/2}$ (Å)	$[\eta]$ (dl g^{-1})
13	Cyclohexane	35.4	43.8*	2200	5.5
13	Benzene	40.0	43.5†	4800	67.7
18	Cyclohexane	35.4	27.4*	1600	4.4
18	Benzene	40.0	26.8†	3500	36.5

* Value derived from light-scattering measurements. † Value derived from ultracentrifugation measurements.

preparations showed an extreme susceptibility to chemical degradation if trace amounts of THF were present. This effect apparently occurred even when the dry sample was stored because the viscosities of freshly prepared benzene solutions continually decreased with the storage time of the dry sample. We did not investigate whether chemical or mechanochemical effects are predominant. Instead, we found that the degradation could be avoided if we eliminated all traces of radical-forming materials like THF. In later studies mixtures of nitroethane and nitropropane were used to form a density gradient mixture for sedimentation equilibrium. Here again, in the presence of these seemingly inert solvents there occurred degradation of the polymer upon storage.

Large shear forces were avoided whenever it was possible. For example, we placed solutions in light-scattering and ultracentrifuge cells by pouring through as large a tube as possible. Also solutions were made by gentle hand shaking and agitation over a period of 1 to 3 weeks, depending on the polymer concentration and the goodness of the solvent. By means of these pouring and mixing techniques, it was possible to obtain reproducible results in the viscosity, light-scattering, and ultracentrifugation measurements.

A low-angle, light-scattering photometer with an optical design of McIntyre and Doderer (*8*) was used for the light-scattering measurements. However, this instrument was not an absolute light-scattering photometer and all measurements had to be made against scattering standards.

We eliminated the need for filtration and sample solutions by using a specially designed cylindrical light-scattering cell containing a dust trap on the bottom. This cell could be placed directly in a centrifuge and the sample optically cleaned by centrifugation. All samples were centrifuged at 4000*g* for 2 hours. The use of this cell eliminated any possibility of sample degradation. The values of the weight-average molecular weight (M_w) and root-mean-square radius of gyration ($\langle S^2 \rangle_z^{1/2}$) in a theta solvent (cyclohexane) and a good solvent (benzene) are given in Table 1. The theta temperature at which the second virial coefficient $A_2 = 0$ for cyclohexane was found to be 35.4°C.

All sedimentation equilibrium experiments were carried out in an analytical ultracentrifuge (Beckman model E) with an An-J equilibrium rotor. Cyclohexane was used as a solvent for the sedimentation equilibrium experiments. These measurements were made at 37°C, slightly above the theta temperature. The minimum speed of 800 rev/min was used in order to achieve the largest accuracy in the determination of both M_w and M_z (the z-average molecular weight). In order to operate at even this minimum ultracentrifuge speed, it was necessary to use column lengths of 1 to 2 mm for the solutions. Some 3 to 4 weeks were required for the cells containing samples of highest molecular weight to come to equilibrium. In all cases the speeds were cut back to 800 rev/min after an initially higher speed. The ratios of M_z to M_w for samples 13 and 18 were 1.15 and 1.18, respectively. All experiments were carried out at three concentrations and the values of M_w and M_z were obtained by extrapolating the apparent molecular weight M_{ap} to zero concentration by Fujita's method (*9*). The values of M_w reported in Table 1 were within 3 percent of the values from light-scattering measurements in cyclohexane.

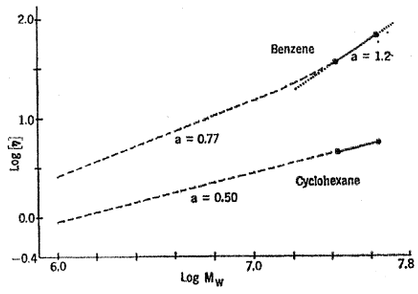

Fig. 1. Logarithmic plot of $[\eta]$ versus M_w for polystyrene fractions in a theta solvent (cyclohexane at 35.4°C) and a good solvent (benzene at 40°C); a is the exponent in Eq. 1. The dotted line and a value for the good solvent are based only on the two data points shown.

Intrinsic viscosity $[\eta]$ measurements were made in a couette viscometer designed by Zimm and Crothers (*10*) with a specially designed drive. The shear rate employed was less than 0.1 sec^{-1}, which can be taken as zero shear since we could not observe any changes in $[\eta]$ from a shear rate of 0.03 to 0.09 sec^{-1}. The solutions were poured directly into the viscometer holder through a large-bore entry tube to prevent the shear degradation that had been observed in earlier experiments in which a capillary inlet tube was used. All concentrations were below 10^{-5} g ml^{-1} so that the relative viscosities were always below 1.2. The values of $[\eta]$ in cyclohexane and benzene are shown in Table 1. The time needed to reach equilibrium after pouring was ½ hour in each case. The extremely dilute solutions were below the critical concentration, thus ensuring that chain entanglement problems were absent.

In order that the results be meaningful, it is mandatory to make absolute measurements of molecular weight rather than relative measurements relying on extrapolations from the literature on lower molecular weights. Figure 1 shows the familiar Mark-Houwink logarithmic plot of $[\eta]$ versus M_w. The dashed lines show the well-established linear behavior above a molecular weight of 10^5 expected according to present-day polymer theories. For the theta solvent cyclohexane at 35.4°C, the Mark-Houwink relation remains valid even for the samples with the highest molecular weight. However, when a good solvent, having a positive excluded volume, is used, there appears to be no unique asymptotic value for the Mark-Houwink equation

$$[\eta] = KM_w^a \qquad (1)$$

where K is a constant. For a good solvent the exponent a changes from its usual high-molecular-weight value of 0.77 to 1.2. (In view of these results, all investigators using GPC calibration curves should be aware of possible errors when materials of higher molecular weight are analyzed.)

Henceforth, biological and polymer chemists can look forward to a greatly expanded range of molecular weights for future studies of linear, flexible polymer molecules. Chain degradation is a problem, but it can be avoided by very careful solvent purification and reasonable handling and storage procedures. Until more is known about the effect of excluded volume at these high molecular weights, the extension of the Mark-Houwink equation to high molecular weights, particularly for biological polymers, should preferably be done for data in theta solvents.

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Gamma-Aminobutyric Acid: Role in Primary Afferent Depolarization

Abstract. *The effects of putative transmitters on the primary afferent terminals were studied in the magnesium-treated, isolated spinal cord of the frog. Gamma-aminobutyric acid and glutamic acid reversibly depolarized primary afferent terminals and increased their excitability, whereas glycine produced weak and variable effects. Bicuculline and picrotoxin, which reduce primary afferent depolarization, reversibly antagonized the gamma-aminobutyric acid–mediated responses but had little effect on those produced by either glutamic acid or glycine. The glutamic acid- and the gamma-aminobutyric acid–induced depolarizations remained in the absence of external chloride but disappeared in the absence of external sodium. These results support the hypotheses that gamma-aminobutyric acid is the transmitter mediating the synaptic depolarization of primary afferent terminals and that sodium is the predominant ion involved.*

The prolonged inhibition of the monosynaptic excitation of motoneurons by orthodromic volleys in muscle and cutaneous afferent fibers is generally attributed to a presynaptic depolarization of the primary afferent terminals that presumably decreases both the amplitude of the presynaptic action potential and the quantity of transmitter released (*1*). The depolarization of the primary afferent terminals (primary afferent depolarization) is electrotonically conducted along the dorsal root where it can be recorded as the dorsal root potential (*1, 2*). The depolarization and the coincident increase in excitability of the terminals suggests that the last step in the generation of primary afferent depolarization is mediated by an excitatory transmitter. Since picrotoxin and bicuculline reduce the dorsal root potential (*3–5*) and selectively antagonize synaptic events mediated by gamma-aminobutyric acid (GABA) (*5, 6*), GABA (or a closely related substance) has been proposed as the transmitter mediating primary afferent depolarization (*1*). However, in all other systems, GABA is exclusively involved in inhibitory events, decreasing excitability by increasing subsynaptic membrane conductance, predominantly to chloride (*7*). We have used the isolated spinal cord of the frog to investigate this paradox since this preparation is suitable not only for studying the action of putative transmitters on primary afferent terminals (*8*), but also for studying the ionic mechanisms underlying these actions. We now report (i) that GABA mimics the action of the natural transmitter by depolarizing and increasing the excitability of primary afferent terminals, (ii) that this action is selectively blocked by both picrotoxin and bicuculline, and (iii) that it is de-

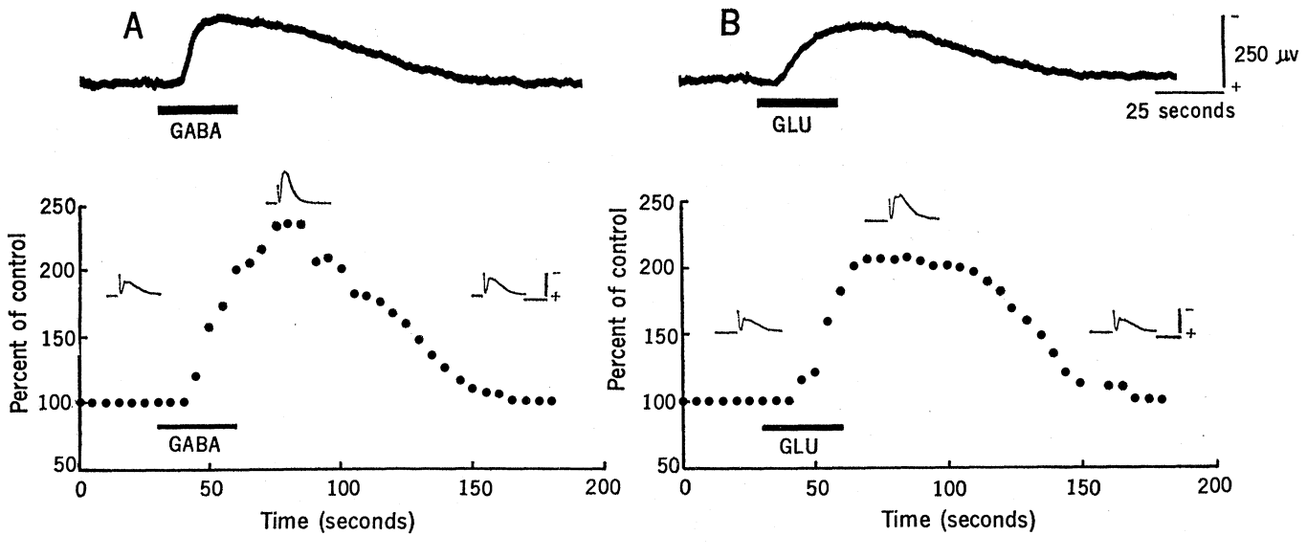

Fig. 1. Gamma-aminobutyric acid and glutamic acid increase the excitability of primary afferent terminals and depolarize the dorsal root of the isolated spinal cord of the frog. Synaptic activity was eliminated 2 hours prior to the above records by the addition of 20 m*M* $MgSO_4$ to the perfusing Ringer solution. The upper trace in each figure is a d-c recording of the polarization level of the dorsal root. The lower part of each figure is a graph of the size of the antidromically conducted dorsal root volleys (recorded a-c every 5 seconds) before, during, and after application of drugs. The insets are examples of these volleys. The excitability testing and the d-c recordings were made within 5 minutes of each other. (A) Application of GABA ($5 \times 10^{-3} M$) for 30 seconds (solid bar, upper trace, and lower graph) depolarizes the dorsal root and increases primary afferent excitability with a similar time course. (B) Application of glutamic acid ($5 \times 10^{-3} M$) for 30 seconds (solid bar, upper trace, and lower graph) also depolarizes the dorsal root and increases excitability with similar time courses. For insets in (A) and (B), vertical calibration is 1 mv and horizontal, 1 msec.