tenuis (*4*), and it was shown that brief irradiation with red or green light was sufficient to potentiate dark synthesis of the appropriate biliprotein. In a series of alternating red and green irradiations the effect depended only on the quality of the last irradiation, which demonstrates multiple photoreversibility of red by green and vice versa. The effect was thought to be due to photointerconversion and photodestruction of precursors of the biliproteins.

The other response is a change from an aseriate (single cell) to a filamentous growth habit in *Nostoc muscorum* A. in dark-grown cultures invoked by a brief irradiation with red light. The effect of red was reversed by a brief irradiation with green light (*5*). The action spectra for this response resemble those for chromatic adaptation in *Tolypothrix* (*6*), the long-wave action maxima being nearly identical for both taxa. *Nostoc* does not chromatically adapt.

While conclusive proof is not yet available, the resemblance of the absorption spectra of the new pigment (Fig. 1) to action spectra for photomorphogenesis in *Nostoc* and chromatic adaptation in *Tolypothrix* suggests that it plays a directive role in both processes. A model for the action of the photoreversible pigment, formulated after familiar models for the phytochrome action, appears in Fig. 2. The multiple arrows indicate that the final display observed is not necessarily a direct result of pigment action. The photomorphogenic phenomenon, for instance, is probably a much more indirect result of photoconversion than is control of biliprotein synthesis. The latter process is relatively rapid and will be of interest in the study of control of the synthesis of specific proteins [phycocyanin and phycoerythrin are serologically unrelated (*7*)] by light, which presumably chronologically precedes the appearance of the photomorphogenic phenomena. The model shows both forms of the pigment as being biologically active; it could as well be formulated to show only one active form, the absence of which allows a given differentiative chain of events to proceed. This would be in closer conformity to proposed models for phytochrome action.

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Stimulation by Phagocytosis of the Deiodination of L-Thyroxine in Human Leukocytes

Abstract. *Intact human leukocytes actively deiodinate* L-[^{131}I]*thyroxine, producing mainly inorganic* ^{131}I *and chromatographically immobile* ^{131}I*-labeled origin material. When phagocytosis is induced, the deiodination is enhanced, a suggestion that deiodination in mediated by a peroxidase–hydrogen peroxide system.* L-*Thyroxine can serve as a source of iodine for iodination reactions within the leukocyte.*

We have observed that leukocytes isolated from the blood of rhesus monkeys inoculated 6 hours previously with viable *Diplococcus pneumoniae* display an enhanced ability to deiodinate L-thyroxine (T_4) in vitro (*1*). We suggested that this phenomenon might be related to the phagocytosis of the organisms by the leukocytes because: (i) phagocytosis is accompanied by a release of myeloperoxidase and by a metabolic burst that results in the generation of hydrogen peroxide (*2*); and (ii) the deiodination of T_4, at least in several tissues of the rat, appears to be mediated by a peroxidase–hydrogen peroxide system (*3*). Accordingly, we examined the influence of induced phagocytosis on T_4 deiodination by human leukocytes.

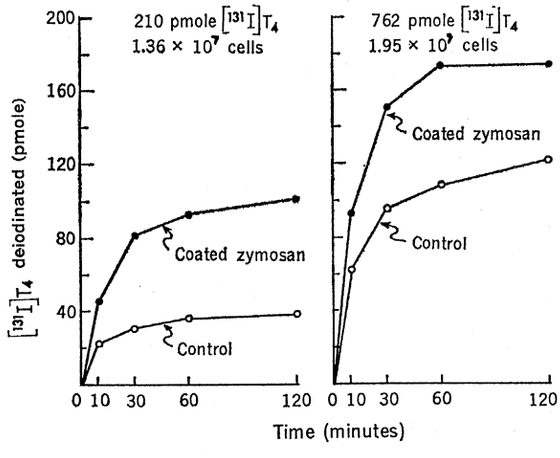
Leukocytes were isolated from normal human blood, with heparin as the anticoagulant (*4*). We used plasticware or siliconized glassware (Siliclad) throughout the experiments. The blood was allowed to sediment at 6°C in a solution of dextran in saline, and the supernatant layer containing the leukocytes was collected and centrifuged. The erythrocytes remaining in the leukocyte pellet were lysed by exposure of the pellet to hypotonic conditions for 30 seconds; the leukocytes were collected by centrifugation and washed with normal saline. We recovered about 50 percent of the leukocytes from the original blood; approximately 90 percent of these were neutrophils with virtually no contaminating erythrocytes. The leukocytes were suspended at a concentration of 1 to 2×10^7 cells in 0.5 ml of Krebs-Ringer phosphate buffer containing 10 m*M* glucose (KRPG), *p*H 7.4. Zymosan, the insoluble polysaccharide residue of the cell wall of yeast, was used as the particulate material for phagocytosis (*5*). The zymosan was coated with plasma by homogenizing 5 mg of it in 1 ml of fresh plasma, was centrifuged and washed twice with KRPG to remove residual plasma, and was then suspended in KRPG to provide a concentration of 0.5 mg/0.1 ml. The leukocyte suspension (0.5 ml) was added to siliconized Erlenmeyer flasks containing [^{131}I]T_4 and either 1 ml of KRPG alone, or 0.9 ml of KRPG and 0.1 ml of coated zymosan. Flasks were also prepared without leukocytes to serve as tissue-free controls. All flasks were prepared in duplicate and were incubated at 37°C in air, in a metabolic shaker. Samples (50 μl) were withdrawn from the reaction mixtures after 10, 30, 60, and 120 minutes of incubation, and were transferred to tubes containing 50 μl of a 15 percent solution of human serum albumin containing carrier T_4, carrier iodide, and propylthiouracil to stop the reactions. The mixtures were then subjected to ascending chromatography on filter paper strips in a solvent system of butanol, acetic acid, and water (*6*). This system permits detection of inorganic iodide and chromatograph-

ically immobile origin material, but not of L-triiodothyronine. The relative proportions of the former two products were quantitated by cutting out the radioactive zones on the filter paper strips, with the aid of radioautograms, and counting them in a well-type scintillation counter. The results obtained from the mixtures containing leukocytes were corrected for spontaneous deiodination by subtracting the results of the corresponding tissue-free controls. The latter were sometimes slightly higher in the presence of coated zymosan, but did not exceed 9 percent of the added $[^{131}I]T_4$ after 120 minutes of incubation. The results obtained with leukocytes killed by heat were essentially the same as those in tissue-free controls.

In contrast to two other findings (*7*), we found that intact human leukocytes did actively deiodinate $[^{131}I]T_4$. The presence of coated zymosan in the reaction mixture was associated with a great increase in the deiodination of $[^{131}I]T_4$ (Fig. 1). Zymosan that had not been coated with plasma was accompanied by little, if any, stimulation of deiodination. This raised the question of whether the stimulation of deiodination resulted from the metabolic burst that accompanies phagocytosis or from the facilitated entry of $[^{131}I]T_4$ into the cell as a result of its binding to the coated zymosan particles. Evidence against the latter was provided by two observations: (i) a comparable stimulation of deiodination occurred in the presence of zymosan that had been coated with plasma devoid of T_4-binding globulin; and (ii) zymosan, even when coated with normal plasma, did not retard the rate of transfer of $[^{131}I]T_4$ from a dialysis sac into a solution of KRPG, and, at equilibrium, the dialyzable fraction of $[^{131}I]T_4$ was virtually identical to that predicted if binding were absent in the dialysis sac.

Pincus and Klebanoff (*5*) and Klebanoff (*8*) have reported that phagocytosing leukocytes take up inorganic iodide and fix it to ingested particles or organisms in a form that is precipitable by trichloroacetic acid. This iodination reaction, which might play a microbicidal role, is catalyzed by myeloperoxidase in the presence of hydrogen peroxide that is generated either by the ingested organism itself or by the phagocytosing leukocyte. We therefore compared the effects of coated zymosan on the uptake by leukocytes of $[^{131}I]T_4$ and of inorganic ^{125}I, and on the generation of chromatographically immobile origin material, representing fixed radioiodine, from these labeled precursors. In this experiment, we added inorganic ^{125}I, enriched with potassium iodide to provide an iodine concentration identical to that of the $[^{131}I]T_4$, to the reaction mixture; additional duplicate flasks were prepared containing inorganic ^{125}I enriched with potassium iodide without $[^{131}I]T_4$. The reaction was stopped after 60 minutes of incubation by the addition of 0.5 ml of a 15 percent solution of human serum albumin containing carrier T_4, carrier iodide, and sodium metabisulfite. The leukocytes and, in the tissue-free controls, the coated zymosan particles were collected by centrifugation of the reaction mixture; the pellets were washed twice with normal saline, and were suspended in a small volume of human serum albumin containing carrier T_4, carrier iodide, and sodium metabisulfite. The suspensions were sonicated, counted against a standard prepared from the corresponding reaction mixture, and chromatographed. The results obtained for the uptake of radioiodine by leukocytes in the presence of coated zymosan were corrected by subtracting the results obtained with coated zymosan alone.

Analysis of the sonicated leukocyte suspensions revealed a greater total uptake of radioiodine derived from $[^{131}I]$-T_4 than from an equimolar concentration of inorganic ^{125}I, even when the latter was present separately (Fig. 2). This indicated active binding of T_4 by intracellular components, as the bulk of the radioiodine taken up was in the form of T_4. The presence of coated zymosan increased the uptake of radioiodine derived from both labeled precursors, but this increase was relatively much greater for inorganic ^{125}I. Chromatographically immobile origin material comprised a small proportion of the total uptake of radioiodine derived from both precursors. The presence of coated zymosan increased the generation of origin material, and again this increase was relatively much greater for inorganic ^{125}I. However, since the total uptake of radioiodine derived from inorganic ^{125}I was much less than that derived from $[^{131}I]T_4$, the amount of chromatographically immobile origin material generated from inorganic ^{125}I was less than that generated from $[^{131}I]$-T_4.



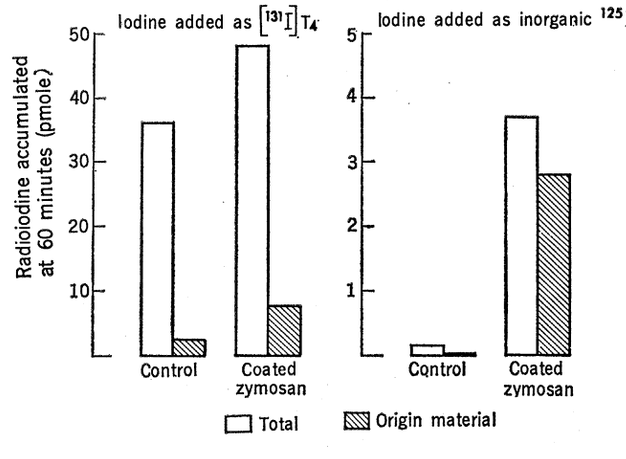


Fig. 1 (left). Effect of phagocytosis of zymosan coated with plasma (*coated zymosan*) on the deiodination of $[^{131}I]T_4$ by intact human leukocytes. Fig. 2 (right). Comparative effects of phagocytosis of zymosan coated with plasma (*coated zymosan*) on the uptake by human leukocytes of radioiodine (627 pmole) derived from equimolar concentrations (with respect to iodine) of $[^{131}I]T_4$ and inorganic ^{125}I and on the generation of chromatographically immobile origin material, representing fixed radioiodine. Leukocytes, 1.30×10^7 cells.

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.