studies presents no special problems owing to the clean separation and sharp resolution of the molecular species.

Table 1 gives the results of the quantitative analysis of the various inorganic phosphate compounds inside and outside the cells after UV irradiation. The amounts may be compared to those in nonirradiated cells before irradiation where the phosphates under discussion represent 74 percent of the total phosphate extracted and 69.8 percent of these is orthophosphate. Figure 1 shows that phosphate leakage is negligible in nonirradiated cells.

The total inorganic phosphate in the system does not differ significantly from that of the nonirradiated cells although irradiation causes loss of all inorganic phosphate species into the medium to the extent of 9.3 to 16.7 percent. This is in contrast to the situation for organic phosphates where certain ones "leaked" selectively from irradiated cells (*1*). Pyrophosphate is the only individual phosphate which shows a significant change in the total amount present in the system after irradiation. The amount of pyrophosphate in the cells plus the suspending medium is approximately 2.5 times that of the control cells. Normally, pyrophosphate represents 4.5 percent of the total inorganic phosphate in the yeast cell. After irradiation, this value is increased to about 11 percent. Katchman and Fetty (*5*) reported that 0.4 mg of phosphorus of high molecular weight polyphosphate was found in *S. cerevisiae* during the logarithmic growth phase and approximately 0.2 mg during the plateau phase. Thus, sufficient polyphosphate is present so that degradation of high molecular weight polyphosphate could account for the increase in pyrophosphate without causing changes in measured total inorganic phosphate.

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## Microstructure of Polymers by Tritium Autoradiography

Abstract. *Polystyrene, polypropylene, and polyethylene, containing tritium-labeled additives and crystallized in thin films, were examined with autoradiographic stripping film. In the structures formed by isotactic polystyrene, tritiated atactic polymer concentrated in specific patterns in the outer regions. Polypropylene spherulites showed marked differences in the distribution of the tritiated additive, dilauryl thiodipropionate, whenever their optical properties also differed. Autoradiographs of polyethylene spherulites containing low molecular weight tritiated polyethylene exhibited concentric ring patterns similar to those observed on viewing the polymer film in a polarizing microscope.*

The microstructure of polymers, especially their crystalline forms, has been investigated by a variety of techniques (*1*). We now report the application of an additional tool, tritium autoradiography, which has unique potential for studying polymer microstructure. Special attention is given to spherulite structures, often found on crystallizing such polymers as polystyrene, polyethylene, and polypropylene.

When an impure material is crystallized, impurities are excluded from the crystalline network. We have utilized this principle in our work. The "impurity," a small amount of a tritium-labeled additive, is deliberately incorporated in the polymer. On annealing from the melt, the additive is rejected from certain areas of the crystallizing polymer and deposited in others. Distribution and localization of the labeled additive is determined with autoradiographic stripping film. The resultant film, examined microscopically, reveals information on microstructure and polymer crystal morphology.

The main advantage of tritium in autoradiography is the high resolution obtainable. Due to the low energy of its beta radiation, the effective range in photographic emulsion is less than 1 $\mu$ (*2*). Therefore, radioactive regions can be easily distinguished in a specimen at separations of less than 2 $\mu$.

Three polymer systems have been studied: polystyrene, polypropylene, and polyethylene. Appropriate tritium-labeled additives were synthesized for each system by conventional chemical methods. Tritium concentrations were made sufficiently high to give good autoradiograms in 3 to 20 days, depending on the material and its concentration in the samples.

For studies with isotactic polystyrene, the stereoisomeric modification was prepared with a tritium label. Acetophenone was reduced with tritium-labeled lithium borohydride (*3*) to give tritiated $\alpha$-methyl benzyl alcohol. Catalytic dehydration of this alcohol with sulfosalicylic acid at 150°C produced styrene. The labeled styrene was thermally polymerized to tritiated atactic polystyrene. The product, having a specific radioactivity of 180 $\mu$c/mg, was used as the additive for isotactic polystyrene.

For polyproplyene, tritium-labeled dilauryl thiodipropionate was used as the additive. This compound, in nonradioactive form, is often used as an antioxidant in polypropylene. Synthesis was achieved by reducing methyl laurate with tritiated lithium borohydride to give lauryl alcohol-$H^3$. The alcohol was then esterified with thiodipropionic acid to yield the desired additive, tritiated dilauryl thiodipropionate. The product had a specific activity of 140 $\mu$c/mg.

For the work with polyethylene, a fraction of low molecular weight was tritiated by the catalytic reduction of its unsaturated groups with tritium gas. The labeled polymer had a molecular weight (number average) of 320, and a specific activity of 1440 $\mu$c/mg. It differed from the host polymer only in molecular weight and in the absence of unsaturated groups.

Thin films were prepared for autoradiographic examination by depositing small amounts of the polymer-additive mixtures on microscope slides. In some instances, films were deposited by evaporation of solvent from decalin solutions; in others the dry materials were spread between two slides and melted. The deposits were re-melted be-

tween slides and annealed by slow cooling in an insulated system. Suitable specimens were selected for autoradiography by examination under a microscope. Next, the top cover glass was removed and exposures were made by the procedure of Pelc (*4*) on autoradiographic stripping film (*5*). Exposures varied from 3 to 20 days. After development, the emulsion was carefully lifted from the wet slide and remounted on a clear area of the same slide to dry. Photomicrographs were then taken of the autoradiographs, and of corresponding areas in the polymer film, with polarized light (Fig. 1).

The structures in Fig. 1, *A* and *B*, grown from isotactic polystyrene containing 1 percent of tritiated atactic polystyrene, are similar to those observed in polyoxymethylene and called hedrites by Geil (*6*). From an examination of the autoradiograph some deductions about the formation of these hedrites can be made. Not only is the noncrystallizing atactic polymer rejected from the central area of the crystallizing isotactic spherulite, as expected, but primary crystallization is seen to proceed in six geometrically uniform directions from the nucleus. This is followed by a "filling in" of the segmental areas between the projections which forces the labeled atactic polymer to outer areas of the segments and away from the radial projections.

When studying polypropylene we observed many negatively birefringent spherulites described by Padden and Keith (*7*) and classified by them as Type III. The photomicrograph in Fig. 1*C* is typical of the appearance of these structures in polarized light. In this instance 5 percent of tritiated dilauryl thiodipropionate was mixed with the polypropylene. Autoradiographic examination of the same film (Fig. 1*D*) shows a surprising contrast in the distribution of the labeled material in the different types of spherulites. In the Type III structure the additive is concentrated in relatively large deposits, leaving much of the spherulite essentially free of additive. An autoradiograph from the opposite surface of the same film showed a remarkable similarity in the distribution and microscopic shape of the labeled deposits, to the finest detail. The thickness of the film was 25 $\mu$, many times greater than the maximum penetration of the beta radiation. Because of this close similarity in fine structure, it seems unlikely that the effect could be caused by exudation of the labeled material from the polymer to the polymer-glass interface. We therefore conclude that these deposits actually persist through the entire thickness of the spherulite, and apparently are incorporated within defects in the structure.

Obviously, the mode of crystal growth in the formation of the different types of spherulites strongly influences the pattern of rejection and inclusion of impurities within the crystalline network. Fundamental reasons for these effects, however, remain to be elucidated.

Concentric extinction rings are frequently observed in spherulites of polyethylene, and many other polymers, when viewed between crossed nicols (*1*). An example of this phenomenon is given in Fig. 1*E*. Figure 1*F* shows the results of examination of the same film, which was cast from polyethylene containing 1 percent of the tritiated polyethylene of low molecular weight. Exclusion of the smaller molecules from central areas of the spherulites is clearly demonstrated. Superimposed on this effect, however, is a pattern of concentric rings in the latent image caused by the deposition of a portion of the labeled polymer. These rings correspond in periodicity with the extinction rings from the polymer film under polarized light. Attempts to match up the extinction rings with those in the autoradiograph were not entirely successful due to difficulty in locating the exact centers of spherulites. However, our measurements do indicate that the dark rings (deposition of labeled polymer) in the autoradiograph correspond

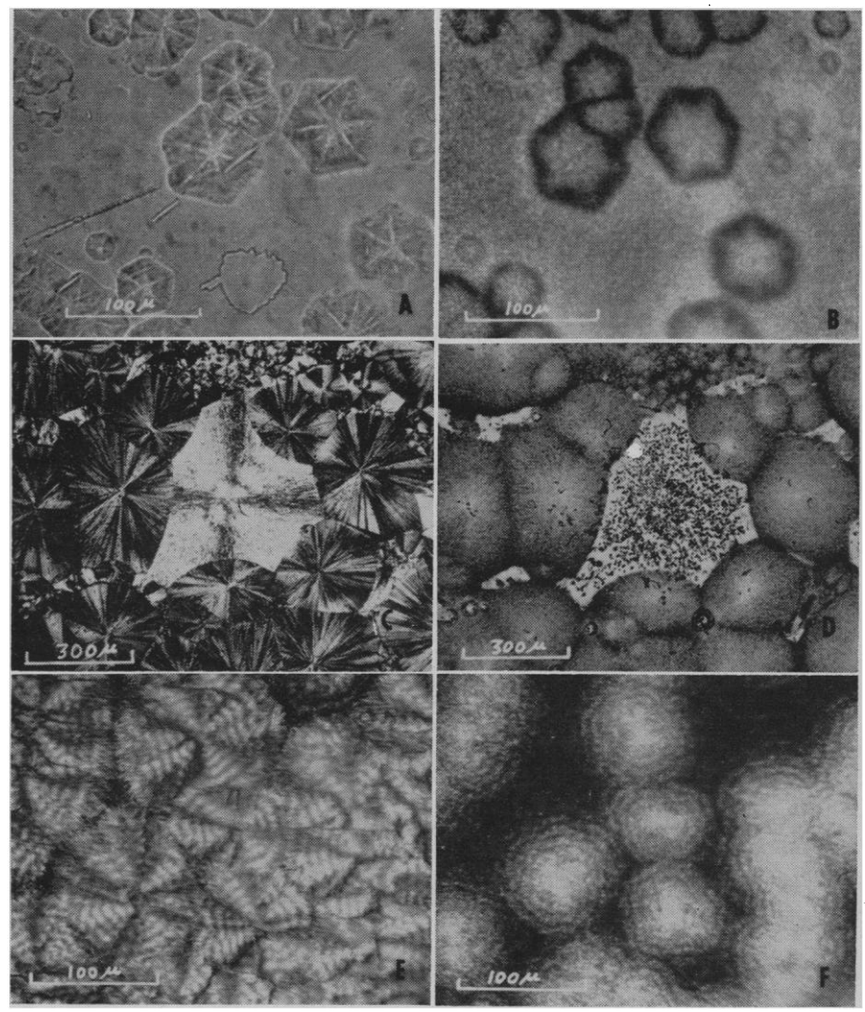


Fig. 1. Photomicrographs of polymer films containing tritium-labeled additives, (left) with polarized light and (right) autoradiographs. (*A* and *B*) Isotactic polystyrene with 1 percent labeled atactic polystyrene. (*C* and *D*) Polypropylene with 5 percent labeled dilauryl thiodipropionate. (*E* and *F*) Polyethylene with 1 percent tritiated polyethylene of low molecular weight.

to light regions in the film under polarized light.

Although discussion in this report has been limited to crystalline systems in which additives were used that are compatible with the polymer, we have also applied the technique to films of crystalline and noncrystalline polymers mixed with additives that are partially or entirely immiscible with the polymer. Distribution, size, and shape of labeled deposits are readily discernible, and such studies should yield valuable information in these kinds of systems as well.

Some decomposition of additive and host polymer due to radiation undoubtedly occurs. However, by the use of a probable "G value" for radiation decomposition, from the work of Tolbert and Lemmon (*8*), it can be calculated that decomposition would amount to less than 1 percent during the course of a typical experiment. To avoid any possible artifacts from such a source, our experiments were always carried out with recently purified additives and freshly prepared mixtures of additive and polymer.

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## Upper Temperature Limit of Life

Abstract. *Samples of microorganisms from the hot springs of Yellowstone National Park have been collected and tested for the ability to utilize radioactive phosphorus. No evidence for growth was found above 73°C.*

Survival of organisms under extreme conditions may depend on isolation of their internal environment from inordinate salt concentrations, *p*H, or even pressure. However, there would be no defense against high temperatures unless the laws of thermodynamics were violated or other sources of energy were utilized. If cells were able to grow at almost boiling water temperatures, it is more likely that the cells would be at the local ambient temperature. The biophysics and biochemistry of such organisms would then be extremely unusual.

The highest temperatures on the surface of the earth (other than volcanoes) are found in the hot spring areas in Yellowstone National Park and also in Japan, New Zealand, and Iceland. Numerous reports of algae and bacteria found in these springs have been published during the last century. Many different people have claimed that these microorganisms were growing at temperatures as high as 89°C (*1*). In the laboratory, however, the highest growth temperatures which have been confirmed are 72° to 75°C (*2*). These experiments might suggest a maximum temperature for growth, and it is therefore of interest to use a metabolic test for growth of organisms reported in the hot springs.

All work was performed at Yellowstone National Park, Wyoming. The area of the lower geyser basin of the Firehole River was examined. Temperature measurements were made with thermistor probes. The highest temperature at which obvious algal mats were found was 73°C. All hot springs and their run-offs above 73°C are perfectly clear. Three springs along White Creek were studied in greater detail. In all springs there was strong convection, and the surface temperature was within 1°C of that at 10-cm depth. Samples were taken from the center of the pools, along with material scraped from the pool walls. Samples (100 ml) were sealed in sterile 1-liter polypropylene bottles containing 150 $\mu$c $P^{32}$ as carrier-free $H_3PO_4$. These bottles were then incubated by floating them in the center of the pools where the samples were collected, and duplicate samples were placed at a single (cooler) point in the run-off of the spring. Other 100-ml samples containing algal mats were collected at that same point in the run-off and treated in a similar manner, being incubated at the point of collection and duplicates in the center of the hot pool.

Three pools were used with temperatures as follows. Pool No. 1 was 69.5°C at the center and 57°C in the run-off. Pool No. 2 was 93°C in the center and 72°C in the run-off. Pool No. 3 was 86°C in the center and 73°C in the run-off.

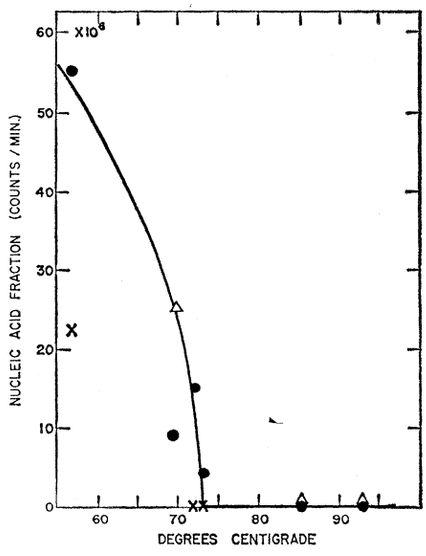


Fig. 1. Incorporation of radioactive phosphorus into nucleic acid at high temperatures. (Circles) Samples incubated at the same temperature as collected; (crosses) samples collected at pool center, incubated in the cooler run-off; (triangles) samples collected in run-off, incubated in hotter pool center.

The boiling point of water at the elevation of Yellowstone Park is 93°C.

Samples were incubated for 48 hours. Cells and other particulate material were removed by centrifugation and washed with 20 ml of tap water. The pellet was then extracted with 5 ml of 5 per cent trichloroacetic acid (TCA) in the cold. After centrifugation, the pellet was treated with 5 ml of 5 percent TCA in a boiling water bath for 20 minutes. The suspension was then chilled in ice and the residue was removed by centrifugation. The residue was resuspended in 2 ml of concentrated HCl. Aliquots of all samples were plated on stainless-steel planchets and evaporated to dryness. The radioactivity determinations were made with an end-window Geiger-Muller tube and scaler.

Any radioactive phosphorus which had been incorporated into the nucleic acids of growing cells would have been extracted in the fraction soluble in hot TCA. This fraction was therefore used as a measure of the growth or metabolism of the cells at each temperature. The results are shown in Fig. 1. It is clear that no evidence for active growth was observed above 73°C.

The explanation of the temperature limitation is unknown. Present concepts