

a high "noise" level, although such a high background is not found in similar systems derived from other rabbit organs. Conceivably, this observation could suggest that two different mechanisms of synthesis were involved. One mechanism is directed by ribonuclease-sensitive messenger RNA, and the other is insensitive to ribonuclease and puromycin. The latter mechanism could be similar to certain peptide synthetic systems discovered in bacteria (14). Such a mechanism would allow for the genetic control of the synthesis of the "backbone" or structural γ -globulin, whereas the synthesis of the antibody-combining sites could have other control mechanisms. Burnet (15) has hypothesized that the combining site of an antibody may be considered as a separate small chain under independent genetic control. Support for the idea of fixed and mutable portions of the γ -globulin molecule has come from two-dimensional peptide patterns of L-chains (16). Other explanations for the behavior of spleen cell-free systems could be formulated, but more data is needed to choose between the several possibilities.

Extraction of RNA with template activity from spleen and lymph nodes of immunized rats has been reported (17). Whether this material will direct the synthesis of γ -globulin is not yet known. A low-molecular-weight RNA has been isolated (18) which appears to convert nonimmune lymphoid cells to antibody-forming cells, and may be in the form of an antigen-RNA complex (19). An evaluation of the function of many cellular constituents, including RNA, membranes, enzymatic factors, mitochondria, and antigen from antibody-producing cells would appear to be necessary to formulate a working scheme for antibody synthesis. The spleen cell-free system may provide an in vitro method for dissecting the effects of some of these materials, and for evaluating the significance of genetic (selective) and environmental (instructional) factors.

KURT H. STENZEL
ALBERT L. RUBIN

Rogosin Laboratories, Department of
Medicine, New York Hospital-Cornell
Medical Center, New York

References and Notes

1. A. H. Coons, *Harvey Lectures Ser.* 54 1957-1958 113 (1959).
2. A. B. Stavitsky, *Advan. Immunol.* 1, 211 (1961).
3. K. H. Stenzel et al., *Proc. Nat. Acad. Sci. U.S.* 51, 636 (1964).

4. M. D. Scharf and J. W. Uhr, *Science* 148, 646 (1965); G. Manner and B. S. Gould, *Nature* 205, 670 (1965); W. L. Norton, D. Lewis, M. Ziff, *Proc. Nat. Acad. Sci. U.S.* 54, 851 (1965).
5. C. J. Wust and G. D. Novelli, *Arch. Biochem. Biophys.* 104, 185 (1964).
6. K. Ogata, S. Omori, R. Hirokawa, *J. Biochem.* 49, 660 (1961).
7. B. A. Askonas, in *Protein Synthesis*, R. J. C. Harris, Ed. (Academic Press, New York, 1961), p. 363.
8. A. L. Rubin and K. H. Stenzel, *Proc. Nat. Acad. Sci. U.S.* 53, 963 (1965).
9. Abbreviations used: ATP, adenosine triphosphate; GTP, guanosine triphosphate; TCA, trichloroacetic acid; RNA, ribonucleic acid; DEAE, diethylaminoethyl.
10. H. A. Sober, F. J. Gutter, M. M. Wycoff, E. A. Peterson, *J. Amer. Chem. Soc.* 78, 756 (1956).
11. G. A. Bray, *Anal. Biochem.* 1, 279 (1960).
12. G. M. Hockwald, G. J. Thorbecke, R. Asofsky, *J. Exp. Med.* 114, 459 (1961).
13. M. C. Ganoza, C. A. Williams, F. Lipmann, *Proc. Nat. Acad. Sci. U.S.* 53, 619 (1965).
14. B. Mach, E. Reich, E. L. Tatum, *ibid.* 50, 175, 1963.
15. F. M. Burnet, Information Exchange No. 5, Scientific Memo No. 6, (1964).
16. K. Titani and F. W. Putman, *Science* 147, 1304 (1965); N. Hilschmann and L. C. Craig, *Proc. Nat. Acad. Sci. U.S.* 53, 1403 (1965).
17. B. Mach and P. Vassalli, *Science* 150, 622 (1965).
18. M. Fishman and F. L. Adler, *J. Exp. Med.* 117, 595 (1964); E. P. Cohen and J. J. Parks, *Science* 144, 1012 (1964); E. P. Cohen, R. W. Newcomb, L. K. Crosby, *J. Immunol.* 95, 583 (1965).
19. H. P. Friedman, A. B. Stavitsky, J. M. Solomon, *Science* 149, 1106 (1965).
20. We thank Professor F. O. Schmitt and Dr. P. F. Davison of the Department of Biology, M.I.T., and Professor L. Levine of Brandeis University for their encouragement; and Mrs. R. Aronson, Miss N. Schechter, Mrs. C. B. Nelson, Miss S. Madero, and Mrs. J. Sullivan for technical assistance. Supported by the New York Heart Association and USPHS grant HE08736.

2 May 1966

Morphology of Nascent Ziegler-Natta Polymers

Abstract. *In the polymerization of α -olefins with heterogeneous Ziegler-Natta catalysts, the polymer is formed directly as long fibrillar units with folded chains. It is proposed that the fibrils are formed by the crystallization of polymer chains growing from the active sites on the catalyst surface, a process which is likened to root growth in whiskers.*

Much attention has been devoted to the study of the morphology of crystalline polymers. Crystallization from the melt and from solution leads to the development of single crystals, spherulites, and other morphological forms in which the lamella with folded chains is the basic structural element (1). Little attention has yet been paid to the morphology of structures formed directly during the process of polymerization. Of particular interest in this regard are the stereospecific

polymers, the majority of which are prepared by carrying out the polymerization on the surface of a solid catalyst (2). On a priori grounds, it might be anticipated that, once formed at the catalytic site, the polymer molecules would become detached from the solid surface and pass into solution in the reaction medium; subsequently, crystallization would lead to the deposition of the now familiar lamellar crystals. We now report what we believe is the first conclusive evidence that the poly- α -olefins, as formed directly in heterogeneous Ziegler-Natta catalysis, have a unique morphology which is quite distinct from that normally observed when they are crystallized from the melt and from solution.

Observations were made on the six polyolefins: polyethylene, polypropylene, polybutene, polystyrene, poly-4-methylpentene-1, and polyisoprene. These polymers were synthesized by conventional techniques (3), the undiluted monomer or diluent monomer mixtures being polymerized with a preformed heterogeneous catalyst made by reacting titanium halide and aluminum alkyl under an inert hydrocarbon. Initially the finely divided catalyst particles were dispersed uniformly throughout the reaction mixture, but as the reaction proceeded the polymer appeared as lumps of swollen gel in which the bulk of the catalyst was concentrated. The reactions were terminated by poisoning the catalyst with methanol, and, after filtration, the solids were washed with a solution of acetone and hydrochloric acid in order to remove the catalyst particles. Suitable solvent treatments removed low-molecular-weight atactic and stereoblock polymers, and finally the specimens were suspended in an appropriate solvent and stored in this condition.

Observations in the optical microscope revealed that in most cases the polymer is formed as discrete hollow particles with a fibrous texture, but in a few instances, depending upon experimental conditions, it could also form as fibrous sheets or webs. As an illustration of the type of structure to which reference is made, Fig. 1, A and B, show globules of isotactic polystyrene suspended in toluene as viewed in the optical microscope between crossed Nicol prisms.

To investigate further the apparent fibrosity of the nascent polymer, it

was dried and replicated by a shadow-transfer method. Examination of the replicas in the electron microscope showed that the polymer is composed of a profusion of fibrils, 200 to 1000 Å in width, and of indefinite length (Fig. 1C).

The fibrils themselves have a fine structure, as indicated by the presence of notches running perpendicular to their length. This feature was more clearly visualized when the globules were disrupted mechanically, and the fibrils so obtained were examined by direct transmission in the electron microscope. The fibrils appear to be composed of platelets, about 100 Å in thickness, stacked like a skewed deck of cards along the fibril axis (Fig. 2).

However, closer examination of the micrographs and detailed considerations of the possible modes of fibril growth suggest that this appearance is illusory and that the helicoidal structure (Fig. 3) may be closer to reality.

In order to obtain information on the disposition of the chain molecules within the fibrils, low-angle x-ray and electron-diffraction experiments were performed. The low-angle x-ray patterns were obtained from coherent films prepared by allowing the globules to dry down and collapse on a glass plate. The patterns showed well-defined reflections in several orders corresponding to a fundamental spacing of the order of 100 Å, in good agree-

ment with the fibril platelet thickness, as determined from the electron micrographs. Evidently, then, the long spacing originates from the periodic arrangement of platelets within the fibrils. The electron diffraction patterns, obtained from fibril preparations similar to that in Fig. 2, showed Debye-Scherrer rings with the specimen normal to the beam. When the plane of the specimen was tilted in an oblique position with respect to the beam, the continuous rings are replaced by pairs of arcs. Calculations based upon these patterns indicate that the chain axis or molecular helix direction is perpendicular, or nearly so, to the plane of the fibril platelets. Since the molecular chain length great-

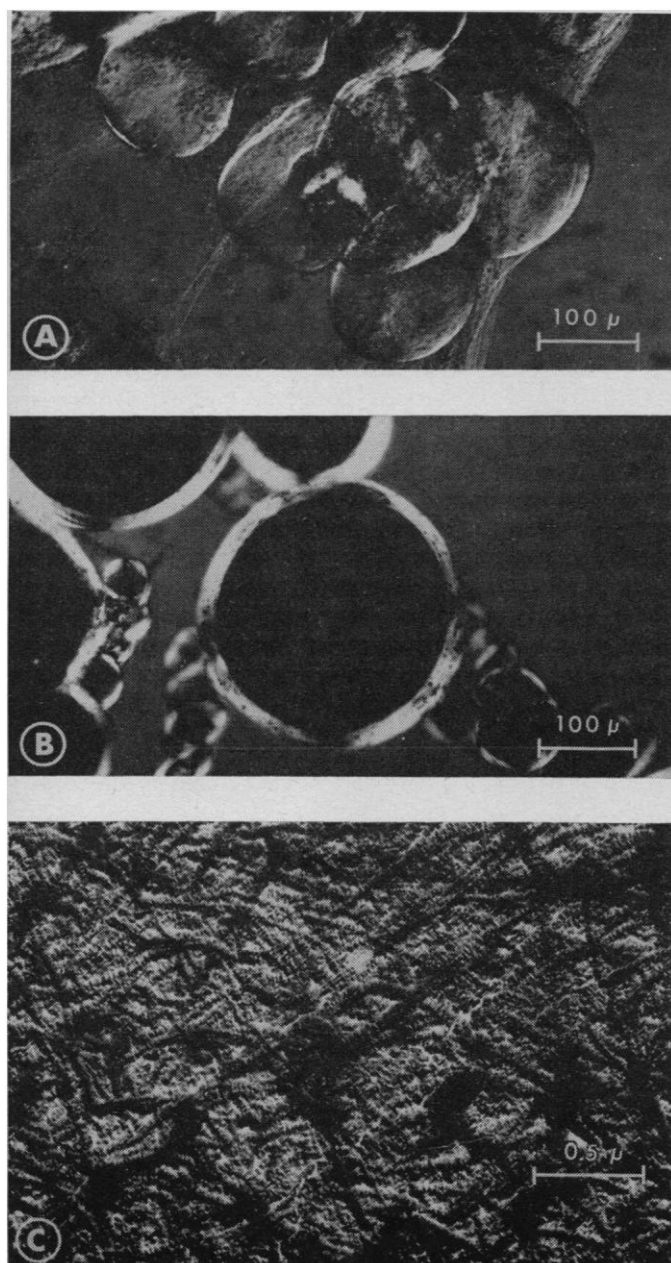


Fig. 1 (left). Photomicrographs showing (A) globules of isotactic polystyrene freed of catalyst and resuspended in toluene (crossed Nicol prisms); (B) a preparation similar to that in A with the focus adjusted to show the skin of the globules (crossed Nicol prisms); (C) a portion of a polystyrene globule which had been dried on a glass slide and replicated (electron micrograph, Pt-Pd shadow cast). Fig. 2 (above). Fibrils similar to those in Fig. 1C, obtained by mechanically disrupting a globule of isotactic polypropylene. Electron micrograph, platinum shadow cast.

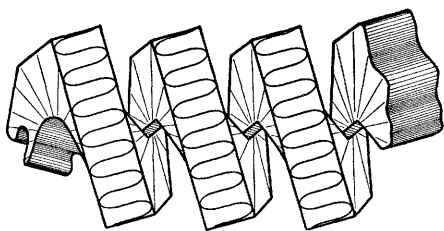


Fig. 3. Schematic drawing of the fibril as a helicoidal structure with folded chains.

ly exceeds the platelet thickness, it seems reasonable to conclude that the fibril platelets are composed of folded chains (Fig. 3).

The phenomenon of the growth of fibrillar crystals with folded chains, as described, has been observed in all six of the polymers studied. It therefore seems to be a general feature of heterogeneous Ziegler-Natta catalysis. It is noteworthy, however, that it apparently does not occur in homogeneous catalysis. For example, a polyethylene sample prepared with the soluble catalyst—dicyclopentadienyl-titanium dichloride—dimethyl aluminum chloride (4)—consisted of the conventional lamellar single crystals.

The significance of these findings may extend beyond their morphological interest. Indeed, the growth of long whisker-like crystals during polymerization with heterogeneous catalysts must be intimately related to the physicochemical processes taking place at the catalyst surface.

The nature of these processes is not fully understood. It is convenient to consider the development of structure in these systems in two stages, namely, the growth of fibrils and their organization into hollow sacs. Experiments by Rodriguez and Gabant (5) suggest that the active sites of an $\alpha\text{-TiCl}_3\text{-Al}(\text{CH}_3)_3$ catalyst are localized along growth spirals on the catalyst surface. Traces of polymer appearing as dots along the spirals have approximately the same width as our fibrils.

It is thus reasonable to suppose that the fibrils are formed by the crystallization of polymer chains growing out from the active sites. We envisage that one end of the fibril is anchored on the catalyst surface and the process can therefore be likened to root growth in whiskers. Many conceptually possible mechanisms of the globulation have been considered, but they all have shortcomings. It should be emphasized that, while the formation of

globules can be suppressed by proper choice of experimental conditions, the observed fibril growth occurred under all conditions in these polymerizations and may therefore be considered as basic to the mechanism of Ziegler-Natta catalysis.

In many stereospecific polymerizations, crystallization takes place with remarkable facility as compared to normal solution crystallization. Whereas isotactic polystyrene will not crystallize from solution in xylene or toluene in any reasonable time (up to several months), it does so virtually simultaneously with polymerization as indicated above. Other polymers show similar behavior. This may indicate that a heterogeneous catalyst, in addition to exerting a strong influence on the

stereoregularity of the polymer, also serves as a "nucleator" by governing the conformation of the polymer as it leaves the surface.

P. BLAIS

R. ST. JOHN MANLEY

Department of Chemistry, McGill University, and Physical Chemistry Division, Pulp and Paper Research Institute of Canada, Montreal, Quebec

References and Notes

1. P. H. Geil, *Polymer Single Crystals* (Wiley, New York, 1963).
2. G. Natta, *J. Polymer Sci.* **16**, 143 (1955).
3. W. R. Sorensen and T. W. Campbell, *Preparative Methods of Polymer Chemistry* (Interscience, New York, 1961).
4. D. S. Breslow and N. R. Newburgh, *J. Amer. Chem. Soc.* **81**, 81 (1959).
5. B. Hartigay, L. Rodriguez, M. Miotto, *J. Polymer Sci.* **35**, 559 (1959); L. Rodriguez and J. A. Gabant, *ibid.* **40**, 123 (1963).
- 12 May 1966

Dormancy Regulation in Peach Seeds

Abstract. *An inhibitor of seed germination was isolated from the integuments of Lovell peach seeds. Evidence from chromatographic analysis, from studies of its absorption of ultraviolet light and from assays on its effects on plant growth, indicate that the inhibitor is, if not identical with, dormin. Termination of rest in peach seeds is correlated with the disappearance of the inhibitor. The effects of the inhibitor are antagonistic to those of gibberellic acid on sections of wheat coleoptiles.*

Studies of growth inhibitors in seeds may be useful in determining the mechanisms controlling rest in hardy plants. In such studies, it is necessary to identify the compounds responsible for inhibition of germination and to correlate their concentrations in the seed with the termination of rest. We now report the isolation of an inhibitor from peach seed integuments, its probable identification, and its relation to rest in peach seeds.

Peach seeds are dormant at the time of fruit harvest and normally require stratification at 2° to 5°C under moist conditions for 10 to 12 weeks to bring about the resumption of growth (1). However, removal of the integuments results in immediate germination of nonstratified seeds (2). The seedlings which develop from these seeds are either dwarfed or normal, depending on the germination temperature (3). Vegis (4), and Wareing *et al.* (5) have reviewed the literature on dormancy in higher plants; considerable reference was made to the complex termed " β -inhibitor" (6) as the possible regulator of dormancy.

Intact peach seeds (200 g) were extracted in four changes of 200 ml each

of distilled water at 0°C for 7 days. The aqueous extracts were combined, acidified to pH 2 to 3 with HCl and extracted in 400 ml of anhydrous diethyl ether. The ethereal fraction was partitioned into acidic and neutral fractions by methods essentially similar to those described by Kefford (7). The acidic fraction contained a substance which inhibited both the elongation of sections of wheat coleoptiles and the germination of wheat and peach seeds. It had a relative flow rate (R_F) value characteristic of the β -inhibitor (6). The inhibitor was located mainly in the outer and inner integuments of the seed and was distributed equally throughout them. Its action was expressed at the micropylar end of the seed where it inhibited radicle elongation, which is the first phase of embryo growth in germination.

The inhibitor is acidic in nature; soluble in water, ether, ethanol, methanol, acetone, and aqueous solutions of Na_2CO_3 ; and was stable at 100°C for at least 10 to 15 minutes. The gross characteristics of the peach seed inhibitor were essentially the same as those described for the β -inhibitor (6).

The acidic fraction and numerous