them, is probably responsible for formation of the authigenous calcite crystals. Such modes of formation are not inconsistent with oxygen isotope data.

Dolomite is associated with calcite in sample Chall 276. Crystals of dolomite of unspecified origin have been observed occasionally in pelagic clays of the Pacific (*11*), and a dolomite-rich layer in contact with a basalt sill has been observed in the experimental Mohole core off Guadalupe; formation of dolomite was related in that case to alteration of the basalt (*12*). Formation of dolomite in Chall 276 could be related to the same hydrothermal-type processes suggested above for the origin of associated calcite crystals. In fact, dolomite should be precipitated directly from warm solutions with a relatively high ratio of Mg to Ca (*13*).

Precipitation of carbonates as a consequence of submarine volcanic activity, based on observations of ancient, now emergent, volcanic formations, has been suggested in the past (*14*).

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Biosynthesis of Gamma Globulin: Studies in a Cell-Free System

Abstract. *Incorporation of C^{14}-amino acids into high-molecular-weight material precipitable by trichloroacetic acid indicates that microsomal cell-free systems, derived from spleens of immunized rabbits, are active in protein synthesis. Protein was made soluble by ultrasonic irradiation of the cell-free incubation mixtures, and low-molecular-weight materials were removed by dialysis and gel filtration. Chromatography and radio-immunoelectrophoresis of this soluble protein fraction reveal C^{14}-labeled protein having several characteristics of gamma globulin.*

Mechanisms of the production of γ-globulin and antibody are not well defined. Immunoglobulins are synthesized primarily in the lymph nodes and spleen by lymphoid cells and plasmacytes. γ-Globulin and antibody are present in these cells (*1*), and the isolated cells produce antibody (*2*). The ribosome-rich endoplasmic reticulum of plasmacytes is thought to be the site of antibody synthesis. Sucrose density-gradient centrifugation of ribosomes isolated from spleen cells suggests that the active synthetic units are single and double ribosomes (*3*). Similar studies of lymph node homogenates have shown that either small or large aggregates may be active in protein production (*4*). Ribosomes isolated from rat spleens have been utilized in cell-free systems and they are active in polypeptide synthesis (*5*). Whereas it has been reported that γ-globulin may be identified as a product of lymph node cell-free systems by immune coprecipitation techniques (*6*), it has not, to our knowledge, been further defined (*7*). We now describe a microsomal cell-free system, derived from rabbit spleens, in which there is incorporation of C^{14}-amino acids into material having several characteristics of γ-globulin.

New Zealand white female rabbits were immunized, their spleens were removed, and the cells were separated and washed essentially as described (*3*). Microsomes and a fraction insoluble at *p*H 5 were prepared by methods used for brain cells (*8*). The fractions insoluble at *p*H 5 were also prepared from rabbit brain and liver. All fractions were frozen at −30°C and used within 24 hours of preparation. The complete incubation mixture for amino acid incorporation contained, in 0.7 ml, 25 μmole of tris, *p*H 7.4; 25 μmole of KCl; 5 μmole of $MgCl_2$; 10 μmole of glutathione; 0.5 μmole ATP (*9*), 0.125 μmole of GTP, 25.0 μmole of phosphoenol pyruvate; 0.03 mg of pyruvate kinase; 5 to 10 μc of C^{14}-leucine (233 mc/mmole); 0.1 to 0.2 mg of microsomes; and 0.2 to 0.4 mg of the fraction insoluble at *p*H 5 from rabbit spleen, liver, or brain. Larger systems, containing up to 0.5 mg of microsomes in 1.4 ml, were prepared for analytical studies. These were incubated with both C^{14}-leucine (233 mc/mmole) and C^{14}-proline (185 mc/mmole) to enhance the specific activity of the nascent protein.

Small systems, and portions of the large ones, were precipitated at 0 and 60 minutes with 5 percent TCA or a mixture of 5 percent TCA and 2.5 percent sodium tungstate. The TCA precipitates were washed (*8*) and layered on Millipore filters (0.45 μ), and radioactivity was determined in a Nuclear-Chicago gas-flow counter. The remainder of the large systems was subjected to ultrasonic irradiation for 2 to 5 minutes in a Branson Sonifier at step 6 (4.5 amp). The soluble fraction, after centrifugation at 110,000*g*, was passed through columns of G25 or G50 Sephadex; the external volume was dialyzed against ten volumes of distilled water at *p*H 5.8, with the water being changed every 12 hours for 3 days. A precipitate, which formed during dialysis, was removed by centrifugation, and the soluble material was lyophilized. In later studies, dialysis was carried out against 0.001*M* tris at *p*H 7.0 (4°C); in this case no precipitate formed, and the material was concentrated, in the dialysis tubing, against dry Sephadex G200. The lyophilized material was reconstituted in small volumes (0.1 to 0.2 ml) of appropriate buffers for chromatography and radio-immunoelectrophoresis. Samples were chromatographed on 2 g of DEAE cellulose (exchange capacity 0.68 meq/g) (*10*), and radioactivity of 0.5-ml portions of the effluent was measured in a liquid-scintillation counter with Bray's counting fluid (*11*).

Optimum incorporation of C^{14}-leucine into material precipitable by TCA is dependent upon microsomes, the fraction insoluble at *p*H 5 (enzymes), ATP, and an ATP-regenerating system. The complete system showed 7950 count/min per milligram of microsomal

RNA. Omission of the brain fraction insoluble at *p*H 5 led to only 40 percent incorporation (3180 count/min). Addition of 1 μg of ribonuclease and 100 μg of puromycin yielded 35 and 40 percent incorporation, respectively. Substitution of the corresponding spleen fraction for the brain fraction insoluble at *p*H 5 led to only 50 percent incorporation (3980 count/min). Omission of microsomes led to less than 10 percent incorporation. The spleen system is similar to microsomal cell-free systems derived from other organs in its relative dependence upon exogenous "*p*H 5 enzymes" and ATP. A 60 precent loss of activity with the omission of these reagents is to be expected, whereas in ribosomal systems the loss of activity is, of course, much greater. On the other hand, the relative resistance of the spleen cell-free system to ribonuclease and puromycin is unusual. When either brain or liver was used as a source of the fraction insoluble at *p*H 5, up to 200 $\mu\mu$mole of C^{14}-leucine was incorporated per milligram of microsomal RNA. The precipitate at *p*H 5 from rabbit spleen homogenates, while not as effective as that from brain or liver, stimulated incorporation of up to 100 $\mu\mu$mole of C^{14}-leucine per milligram of microsomal RNA.

Chromatography on DEAE showed that 5 to 9 percent of the radioactive, TCA-precipitable material was eluted where γ-globulin normally appears (Fig. 1). Coprecipitation experiments (*6*) yielded little information because nonspecific radioactivity in control systems was prohibitively high.

Autoradiographs of dried immunoelectrophoretic and gel diffusion slides were made essentially by the method of Hochwald *et al.* (*12*). Goat antibody to rabbit γ-globulin and rabbit antibody to human γ-globulin were purchased from Hyland Laboratories. Immunoelectrophoretic and gel diffusion slides were washed and dried and exposed to Kodak Royal Pan film or Kodak autoradiographic "no-screen" plates for 4 to 10 weeks and developed for 20 to 25 minutes in Kodak DK60A developer. The slides revealed radioactive precipitin bands which appear in the same area as the band formed between authentic rabbit γ-globulin and its antibody (Fig. 2). The antibody is specific for rabbit 7*S* γ-globulin (IgG) as determined by immunoelectrophoresis against whole rabbit serum. The radioactive band is not as long as the serum γ-globulin band, perhaps an indication of a greater homogeneity of the protein containing the radioactive amino acids. Gel diffusion studies also revealed radioactive precipitin bands in the same area as the bands resulting from reaction of rabbit γ-globulin with antibody to rabbit γ-globulin (Fig. 2). In other experiments, the sound-treated soluble protein fraction was mixed with both rabbit and human serums and subjected to immunoelectrophoresis and autoradiography. Antibody to rabbit γ-globulin was placed in one trough of the immunoelectrophoresis slide and antibody to human γ-globulin in the other. A radioactive band appeared only on the side with antibody to rabbit γ-globulin (Fig. 2). Incubation of γ-globulin with the cell-free mixture in the absence of microsomes failed to produce radioactive precipitin bands (Fig. 2).

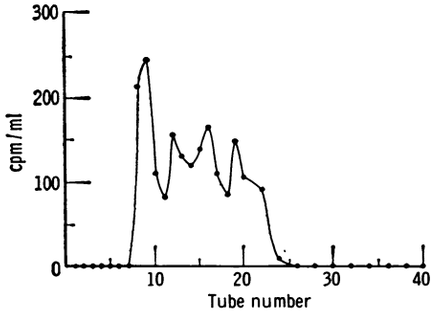

Fig. 1. Chromatography on DEAE-cellulose of soluble material isolated from spleen microsomal cell-free systems after ultrasonic irradiation. The column was equilibrated with 0.02*M* phosphate, *p*H 8.0, and the material was eluted with the same buffer at room temperature. Samples from each tube were removed, and radioactivity was determined in a liquid-scintillation counter.

Microsomal cell-free systems derived from rabbit spleens are thus capable of incorporating amino acids into material which has some important characteristics of γ-globulin. Immunologically specific γ-globulin accounts for only a small portion of the total TCA-precipitable radioactive substance formed, and therefore its identification is difficult. Radio-immunoelectrophoresis appears to be a highly specific technique (*13*) which eliminates many of the difficulties encountered in identifying components of heterogeneous mixtures of C^{14}-labeled polypeptides. In combination with other physical-chemical methods, it may also be applied to the problem of detecting specific antibody in the spleen cell-free system. We deal here with the identification of material with antigenic and chromatographic properties of γ-globulin. Whether specific antibody can be detected in an in vitro system remains to be determined. The biosynthetic mechanism responsible for the incorporation of amino acids into protein in the spleen cell-free system is also unresolved. The relatively high activity of the system in the presence of ribonuclease and puromycin could represent

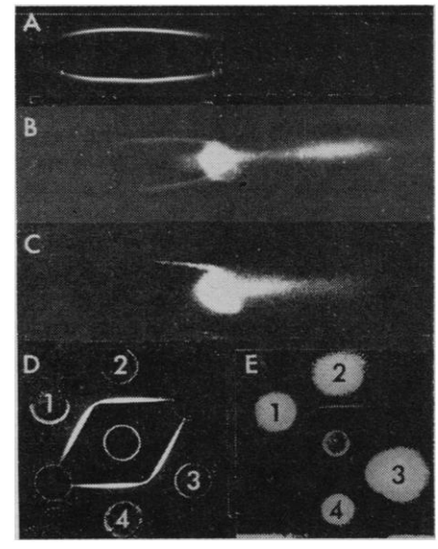

Fig. 2. Radio-immunoelectrophoresis of material formed in the rabbit spleen microsomal cell-free system. Soluble material was released by ultrasonic irradiation of the incubation mixture, and the particulate matter was removed by centrifugation. The soluble fraction was passed through Sephadex G50, dialyzed against distilled water, and lyophilized. (*A*) Stained slide, and (*B*) autoradiogram of the same slide, developed after 8 weeks of exposure. The center well contained a mixture of 3 μl of rabbit serum and 10 μl of the sound-treated soluble material that had been treated with high-frequency sound. Both troughs contained goat antibody to rabbit γ-globulin. The anode is on the right and the cathode on the left. (*C*) Autoradiogram of immunoelectrophoretic slide which contained 10 μl of the sound-treated, soluble material from the cell-free system plus 3 μl rabbit serum and 3 μl human serum. The top trough contained goat antibody to rabbit γ-globulin and the bottom contained rabbit antibody to human γ-globulin. (*D*) Stained slide and (*E*) autoradiogram of gel diffusion preparation. Center well contained goat antibody to rabbit γ-globulin; well No. 1 contained material from a spleen cell-free system with the spleen fraction insoluble at *p*H 5; well No. 2 contained similar material, but with the corresponding fraction from brain substituted for the spleen fraction; and wells No. 3 and No. 4 both contained material from systems incubated without microsomes, but with varying amounts of rabbit γ-globulin.

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 (instructive) factors.

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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