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10. See, for example, A. E. Ringwood, *Geochim. Cosmochim. Acta* **24**, 159 (1961). "Precursors" in this sense, and throughout this paper, refers to materials thought to be physically and chemically similar to those from which less-primitive chondrites derived. The true parental material has, of course, been altered. In using the comparative term "primitive" we mean only that a given meteorite (or group of meteorites) seems to have escaped the effects of a physical or chemical process that left discernible imprints on "less primitive" meteorites: for instance, the presence of glass and clinopyroxene (both believed to be unstable under mild reheating) in a meteorite implies that it was not reheated late in its history; the presence of plagioclase exsolution lamellae in orthopyroxene grains, or of devitrified glass, may mean that a meteorite was reheated late in its history. Thus we would tentatively refer to the first as "primitive" and to the second as "less primitive." Neither "primitive" nor "precursor" implies anything regarding the ultimate origin of chondrites.
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28 April 1966

Subclasses of Human Immunoglobulin A Based on Differences in the Alpha Polypeptide Chains

Abstract. *Antiserum from goats immunized with heavy polypeptide chains from a γA-type myeloma globulin was absorbed with serum from patients with selective absence of immunoglobulin A (γA). The resulting reagents could be used for the classification of 58 γA-myeloma proteins into two distinct antigenic types, respectively called subclasses He and Le. These differences were shown to be related to the heavy (alpha) polypeptide chains and independent of the integrity of interchain disulfide bridges. The γA-immunoglobulin from normal serum appears to consist, for the most part, of molecules with Le subclass specificity.*

The existence of four main classes of human immunoglobulins has gained wide acceptance. One of these, termed the γG-immunoglobulin (IgG immunoglobulin G), is by far the major antibody-carrying globulin in normal serum. The second and third members of the group, called γA- and γM-immunoglobulins (IgA and IgM), are quantitatively less important, whereas the fourth class, known as γD-immunoglobulin (IgD), exists in only trace amounts in normal serum (*1*). The differences between these four types of molecules reside in the structure of their heavy polypeptide chains, which are termed respectively γ-, α-, μ-, and δ-chains. In contrast, all classes of immunoglobulins share the same types of light polypeptide chains, of which two main varieties are known to exist, namely κ- and λ-chains. All six forms of immunoglobulin polypeptide chains occur in a seemingly infinite variety of forms, which constitute the basis of the functional and chemical specificity of antibodies and paraproteins. The γG-class of immunoglobulins may be further subdivided into at least four subclasses (termed Ne, We, Vi, and Ge, or γ_{2a}, γ_{2b}, γ_{2c}, and γ_{2d}) on the basis of structural features of the γ-polypeptide chains, most easily demonstrated by immunological techniques (*2*). Two of these subclasses (We and Vi) are the substrate for the genetical variation known as the Gm system (*3*). A search for the occurrence of similar subclasses among the other types of immunoglobulin polypeptide chains is indicated. Harboe *et al.* (*4*) reported that the γM immunoglobulins could be subdivided into two major types according to immunological characteristics of the heavy μ-polypeptide chains. We can now report that there are at least two different subclasses among the γA-immunoglobulins, based on differences in their α-polypeptide chains.

Six goats and five rabbits were immunized with a variety of antigens, namely whole normal human serum, red cells coated with salivary isoagglutinins (presumed to consist chiefly of γA-type antibodies), three purified γA-myeloma globulins, and purified α-chains from two additional γA-myeloma proteins. Only three among these antiserums proved useful in distinguishing the two subclasses of γA-immunoglobulins here described. All three antiserums were obtained from goats injected with α-chains from a single γA-myeloma protein. Although these three antiserums gave qualitatively identical results, they differed with respect to the intensity of their reactions. Most of our results here presented were obtained with an antiserum which initially reacted with many serum proteins, but could be made almost specific for γA-immunoglobulins by means of absorption with one-tenth its volume of serum from either of two patients with selective γA agammaglobulinemia. The resultant absorbed serums are called *Bm* and *Bc*.

The antiserum *Bm* was allowed to diffuse against five different γA-myeloma serums, as well as against a pool of normal serums, all diluted to approach equivalence (Fig. 1). In their precipitin patterns, two of the myeloma proteins, Wa and He, display some antigenic deficiency with respect to the three remaining myeloma proteins, Le, Ri, and Cl, as well as to the γA-immunoglobulin from pooled normal serum. Among 58 γA-myeloma serums tested, 54 reacted in the same way as Le, Ri, and Cl, that is, their precipitin lines gave a spur over the precipitin lines produced by myeloma serums He and Wa. The lines from all 54 myeloma proteins of the first group showed complete identity with each other when developed with antiserum Bm. The lines from Wa and He also completely fused with each other and with those of two γA-myeloma serums. Hereafter,

γA-myeloma proteins showing the typical deficiency pattern with antiserums Bm or Bc will be said to belong to subclass *He*, whereas the other subclass will be called *Le*.

When antiserums Bm or Bc were absorbed with 0.5 mg of myeloma protein He per milliliter of antiserum, the reactivity against all myeloma proteins of subclass He disappeared, whereas the reaction with all myeloma proteins of subclass Le as well as with normal serum persisted, though weakened. In contrast, absorption of antiserum Bm or Bc with myeloma protein Ri (0.5 mg/ml) removed all reactivity against both subclasses, confirming the antigenic deficiency of subclass He proteins.

There was no correlation between subclass and electrophoretic mobility in agar gel. Attempts to prepare antiserums specific for subclass He have failed so far, even when isolated α-chains were used as the immunizing antigen.

The question of whether the antigenic determinants responsible for the subclass differences were carried by the light or heavy polypeptide chains of the γA-immunoglobulin molecules, was decided in favor of the latter on the following grounds. First, both γA subclasses included representatives of proteins having either kappa or lambda types of light chains. Secondly, the antiserums Bm and Bc had been absorbed with serum from patients having normal amounts of γG- and γM-immunoglobulins, and therefore it could be assumed that antibody activity against both κ- and λ-chains would have been removed from such antiserums. This was strengthened by the fact that both antiserums failed to react with γG-immunoglobulin, when tested by immunoelectrophoresis against normal serum.

Also the fundamental precipitin pattern of Fig. 1 remained unaltered after the antiserum was absorbed with an excess (10 mg/ml) of purified light polypeptide chains from myeloma proteins belonging to either of the γA subclasses. In contrast, absorption of the antiserum by means of purified heavy polypeptide chains (α-chains) from myeloma protein Cl (belonging to subclass Le) abolished all reactivity, whereas absorption with α-chains from myeloma protein He yielded a pattern identical with the one obtained after absorption with the corresponding intact myeloma protein. The purity of the light- and heavy-chain preparations was checked by starch-gel electrophoresis and immunodiffusion tests with appropriate antiserums. Nevertheless, it would be difficult to exclude completely the possibility that the antigenic determinants involved in these subclass differences are due to the existence of some tertiary structure only present when certain types of α-chains are linked to light chains.

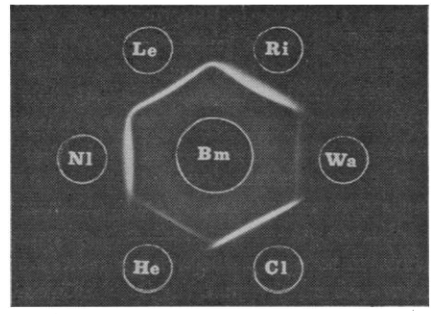

Fig. 1. Precipitin patterns of myeloma proteins. Bm, goat antiserum absorbed with serum of a patient selectively lacking γA (immunoglobulin A). *Le, Ri*, and *Cl* are γA-myeloma serums of subclass Le. *He* and *Wa* are γA-myeloma serums of subclass *He*. *Nl* is normal serum.

It has been demonstrated by Harboe *et al.* (*4*) as well as by Grey *et al.* (*5*) that the precipitin reaction given by certain antigenic determinants of γM-immunoglobulins could be inhibited if disulfide bridges were destroyed by means of mercaptoethanol. In order to assess the part played by disulfide-dependent structures in the immunological differences here observed, myeloma proteins Le and He were partially reduced with mercaptoethanol (0.2*M* in 0.5*M* tris-HCl buffer of *p*H 8.5) and alkylated with iodoacetamide. Of myeloma protein He, two different forms sedimenting as 7*S* and 17*S* components, respectively, occurred in approximately equal amounts in the serum of the patient (*6*); both forms were isolated and studied as such and after reduction and alkylation. These different preparations were compared as shown in Fig. 2. Participation of disulfide-dependent structures in the subclass differences brought out by the preceding experiments is apparently ruled out because (i) the typical spur formation between the precipitin lines of proteins Le and He persisted unchanged after both proteins had been reduced; (ii) after reduction of protein He its antigenic deficiency with respect to the unreduced protein Le likewise persisted; (iii) the disulfide-dependent 17*S* form of protein He (*6*) displayed complete antigenic identity with the 7*S* monomer form of the same protein, and (iv) to these arguments may be added the finding, brought out by starch-gel electrophoretic investigations, that the myeloma proteins of subclasses Le and He displayed a wide variety of polymerization patterns, ranging from a predominance of a 7*S* monomer to a predominance of components of very high molecular size. An incidental finding in the reduction experiments described was that hidden antigenic determinants were brought to light with antiserums Bm and Bc by reduction of disulfide bridges in all γA-myeloma proteins investigated, whatever their α-chain subclass or original molecular size, as illustrated by the spurs of the reduced proteins over their unreduced counterparts (Fig. 2).

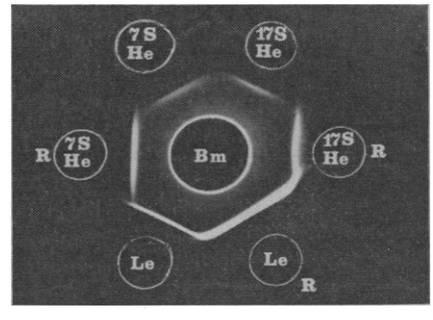

Fig. 2. Comparison of purified myeloma proteins He (7*S* and 17*S*) and Le, analyzed as such and after reduction with 0.2*M* 2-mercaptoethanol and alkylation with iodoacetamide. The reduced preparations are indicated by *R*. Same antiserum as in Fig. 1.

It should also be mentioned that subclass Le appears to be liable to further subdivision, inasmuch as not all myeloma proteins of subclass Le were equally effective in absorbing all reactivity of antiserum Bm. Thus, small amounts of protein Ri removed all reactivity of the antiserum toward all γA-myeloma proteins investigated; however, equivalent amounts of γA-myeloma protein Me, whereas removing all reactivity against subclass He, only absorbed reactivity against some of the proteins of subclass Le. Whether this indicates a third subclass of α-chains is unknown.

In this investigation, the γA-immunoglobulins from pooled normal serum behaved as if they belonged to the Le subclass (Fig. 1). In fact, it seems

more reasonable to assume that He-type molecules do occur in normal serum, but in quantitatively much smaller amounts than Le-type molecules. This at least is the implication of the statistical finding that only 4 out of 58 γA-myeloma proteins belonged to subclass He (*7*).

JEAN-PIERRE VAERMAN
JOSEPH F. HEREMANS
Research Department of Internal Pathology, Cliniques Universitaires Saint-Pierre, Louvain, Belgium

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Acrylonitrile Polymerization in a Miniaturized High-Pressure Optical Cell

Abstract. *A miniaturized high-pressure optical cell has been used to investigate the feasibility of carrying out high-pressure polymerization, wherein the process can be observed both spectroscopically and optically while the polymerization process is occurring. The method has been illustrated by a novel polymerization of acrylonitrile.*

Recently some results have been reported on the high-pressure effect in radical heterogeneous polymerization of acrylonitrile at 50°C (*1*). The pressure effect is unusual in comparison with that of homogeneous radical polymerizations and the heterogeneous radical polymerization of α-methylacrolein (*2*). It seemed interesting to investigate the behavior of acrylonitrile in a miniaturized high-pressure optical cell (*3*), which was shown to be a very useful tool for studying many phenomena at high pressures (*4*).

Mallinckrodt acrylonitrile was used without further purification. Molybdenum foil of 1 mil (0.0254 mm) thickness was used as the gasket material in the diamond cell with a smaller diamond surface of about 0.058 mm^2, as measured by making use of its photomicrograph (250×). The technique for liquid sampling is similar to that described recently (*5*). Infrared spectra of samples in the diamond cell were taken with a Perkin-Elmer 421 infrared spectrometer with a double beam condensing unit. All the experiments were carried out at room temperature (~ 26°C), with the sampling area being viewed through a Leitz microscope supplied with an arrangement for taking photomicrographs.

The pressure "zero-point" was estimated with the melting points of carbon tetrachloride and benzene. With this diamond cell, pressures up to 36 to 40 kb could readily be achieved.

In most runs the pressure was increased and decreased at a speed of about 7 kb per 10 minutes. Under these conditions the crystallization of acrylonitrile in the form of "needles" (phase I) occurs at 28 to 32 kb. At this pressure a new crystalline form (phase II, "plates") starts to grow in a short time, gradually covering all of the field (Fig. 1). A decrease of pressure down to 6 kb does not result in any change. The crystalline form II was viewed through a microscope. At 6 kb the transition II–I occurs (Fig. 2), and at about 5 kb melting takes place. Further decrease of pressure gives rise to the polymerization (Fig. 3), and in about one-half of an hour all of the viewed area is covered by the polymer phase. The polymerization usually starts in a region near 2 to 3 kb.

The great difference in crystallization and melting of acrylonitrile seems to be due to the "superpressing" phenomenon similar to the "supercooling" one. The spectra of liquid acrylonitrile, crystalline form II, and polymer in the region of 2600 to 3300 cm^{-1} are shown in Fig. 4. (The spectrum of crystalline form I looks similar to that of crystal-

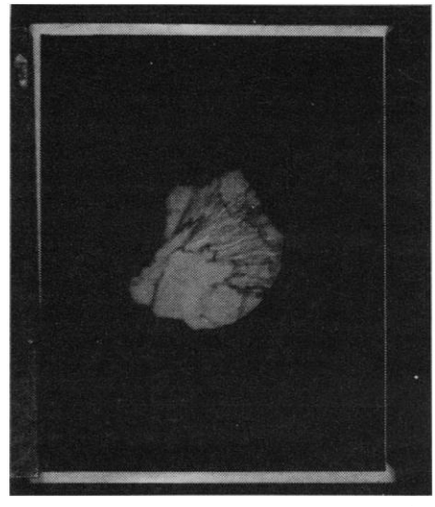

Fig. 1. Crystalline acrylonitrile at about 30 kb and room temperature. Two phases are present; the transition I–II is occurring.

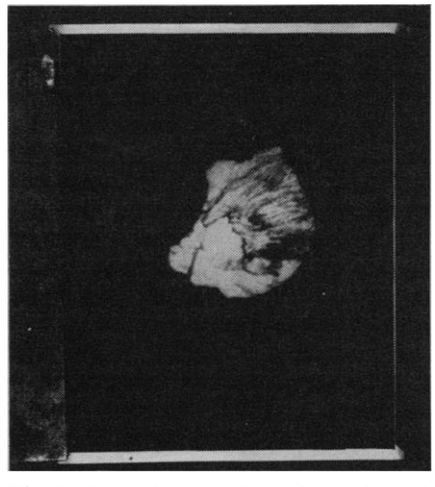

Fig. 2. Crystalline acrylonitrile at about 6 kb. Two phases are present; the transition II–I is occurring.

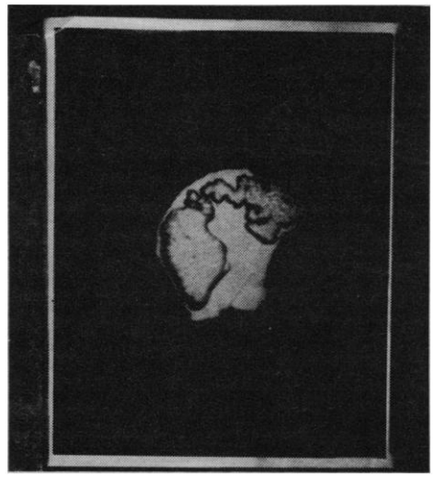

Fig. 3. Polymerization of acrylonitrile in the liquid phase at about 2 kb.