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Localization of Thermoprecipitable Activity to the "Heavy" Polypeptide Chain of a G Myeloma Pyroglobulin.* (30510)

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Myeloma proteins with a characteristic physicochemical property are of special interest for study since antibody activity, which could serve as a tracer, has not been associated with these immunoglobulins. An unusual physicochemical property of a myeloma protein could be used for localization of the activity to submolecular units of the immunoglobulin.

Thermolabile myeloma proteins which precipitate at 56°C and are distinct from Bence-Jones proteins have been termed pyroglobulins by Martin(1). Certain physical, chemical and biologic properties of such a protein have been described(2) and these properties provided a useful means of comparison of this type of myeloma protein precipitate with heat aggregated protein from normal gamma globulin. The precipitate which formed as a result of heating this pyroglobulin reacted with rheumatoid factor, fixed complement and resulted in both Arthus and immediate passive cutaneous type reactions(2). It appeared to be a G myeloma protein on the basis of its sedimentation as a 7S type globulin migrating electrophoretically at the cathodal end of the Ig G peak(2).

Methods and materials. Pyroglobulin serum. Pooled serum was obtained from a patient(2) with multiple myeloma and pyroglobulinemia and kept frozen until used.

Serum electrophoresis. Paper electrophoresis was conducted on a Spinco paper strip apparatus. Agar electrophoresis was done in 1% agar in barbitol buffer, pH 8.6. After electrophoresis, agar strips were heated to 56°C to demonstrate pyroglobulin precipitate and other serum proteins were demonstrated by staining with Thiazine red R.

Immunoglobulin fragmentation. Heavy (H) and light (L) polypeptide chains of pyroglobulin and Cohn Fraction II (F2) (Pentex Corp., Kankakee, Ill.) were obtained by reduction and alkylation. The pyroglobulin serum was fractionated with 33.3% saturated

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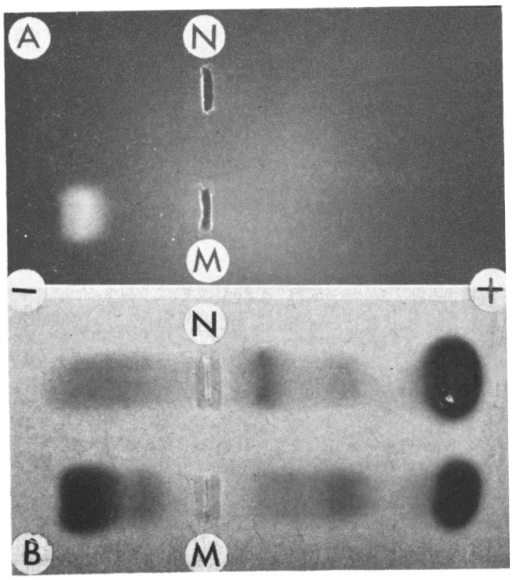

FIG. 1A. Comparison of normal human serum (N) and pyroglobulinemic myeloma (M) serum after electrophoresis in agar and heating to 56°C. (Photographed with transmitted light.) FIG. 1B. Same slide after drying and staining with Thiazine red R. (Photographed with reflected light.)

ammonium sulfate (⅓ SAS)(3). All the pyroglobulin activity was localized to the ⅓ SAS Fraction. This fraction and the normal F2 were reduced in 0.2 M mercaptoethanol, alkylated with 0.38 M iodoacetamide(4) and the H and L polypeptide chains separated on Sephadex G-100.

Starch gel electrophoretic migration of H and L chains of pyroglobulin and F2 was done with formate buffer, pH 3.2, and 8 M urea(5) and in borate buffer with urea-glycine pH 8(5).

Results. Localization of pyroglobulin by electrophoresis. Pyroglobulinemic serum was electrophoresed in agar which was then heated to 56°C, and the characteristic pyroglobulin precipitate was localized to the gamma globulin area (Fig. 1A). The other serum proteins in the pyroglobulinemic and normal sera were compared in the same agar slide stained with Thiazine red R (Fig. 1B). The area of the pyroglobulin precipitate (Fig. 1A) did not appear to equal that of the G myeloma protein (Fig. 1B) because the sensitivity of detection of the protein by staining is greater.

That most of the myeloma peak is pyroglobulin is demonstrated by a paper electrophoretic pattern before (Fig. 2A) and after (Fig. 2B) heating the serum to 56°C and removing the precipitate.

Characteristics of the polypeptide chains of pyroglobulin and F2 immunoglobulin. The elution pattern for the fraction of the pyroglobulin containing serum precipitated by ⅓ SAS which was reduced, alkylated and separated into polypeptide chains on Sephadex G-100 is shown in Fig. 2C. The characteristic pattern of separation into H and L chains by dextran gel chromatography(5) is seen. The type of thermoprecipitable activity which characterizes the pyroglobulin was localized to the first peak of H chain effluent material (Fig. 2C).

The H and L chains of pyroglobulin and F2 studied by starch gel electrophoresis at pH 3.2 are compared with each other and

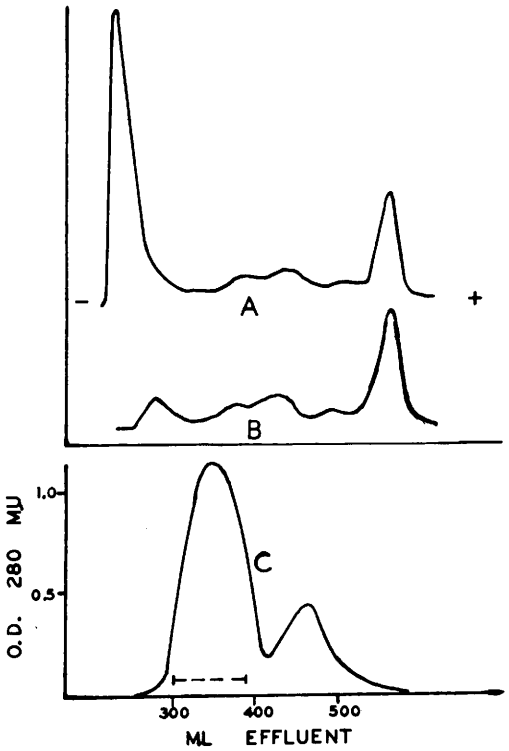

FIG. 2A. Paper electrophoretic pattern of pyroglobulinemic serum before heating. FIG. 2B. Paper electrophoretic pattern of supernatant solution of same serum heated to 56°C. FIG. 2C. Sephadex G-100 effluent pattern of reduced, alkylated myeloma protein. Thermoprecipitable portion of polypeptide chains (– – –).

unreduced myeloma protein (MU) in Fig. 3. At pH 3.2, the myeloma (pyroglobulin) H chain (MH) and F2 H chain (NH) appeared to migrate similarly but the myeloma L (ML) migrated similar to the slow portion of the more diffusely stained normal L (NL) material. At pH 7-8, the myeloma H and normal H migrated in an identical manner as previously described(5). At this pH, most of the myeloma L (ML) migrated in similar manner to just one of the normal L (NL) bands (Fig. 3). Faint traces of other L bands presumably from normal immunoglobulin G are also seen in the myeloma L chain preparation of Fig. 3.

Thermoprecipitability of polypeptide chains. The thermoprecipitable characteristics of pyroglobulin serum, normal F2 and their H and L chains were studied by heating representative aliquots to 56°C and measuring the optical densities at 280 mμ of the precipitates dissolved in 0.1 N NaOH. The H and L polypeptide chains obtained as effluent solutions from dextran gel columns were dialyzed against 0.15 M NaCl buffered at pH 4.5 prior to heating. These conditions were used since H chains of both the pyroglobulin and F2 precipitated without heating at pH 8 unless glycine was added, and it had previously been learned that addition of glycine to whole or fractionated pyroglobulin inhibited the thermoprecipitation at 56°C. The relative amounts of the heat precipitable portions of the H and L polypeptide solutions are compared in Table I.

A significantly larger amount of precipitate was obtained by heating the H chain solution derived from the pyroglobulin than from F2. The H chain precipitates did not dissolve when heated to boiling.

More of the pyroglobulin L chain aggregated when dialyzed against the pH 4.5 buffered saline, accounting for the decrease in total concentration of this myeloma L chain. Both the myeloma L and F2 L partially precipitated at 56°C (Table I). The precipitate formed by heating of both myeloma and F2 L chains to 56°C dissolved at 100°C and reprecipitated when cooled.

Discussion. While the thermoprecipitable proteins described as pyroglobulin are not

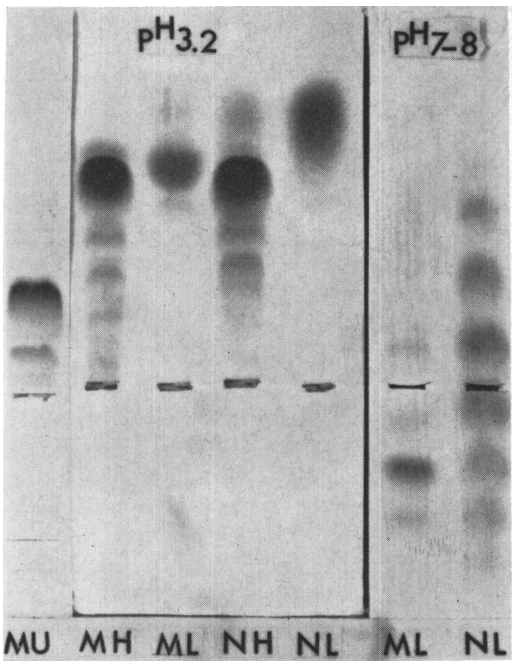

FIG. 3. Starch gel electrophoretic patterns of L and H polypeptide chains at pH 3.2 and 7-8. MU, unreduced 1/3 SAS fraction of myeloma serum; MH, myeloma H; ML, myeloma L; NH, normal (F2) H; NL, normal (F2) L.

common, several patients with this serum abnormality have been reported(1). Such unusual physicochemical properties of a protein provide a marker for comparison with immunoglobulins from normal subjects. The localization of antibody activity to submolecular immunoglobulin units has been done, and, as is presently shown, the characteristic activity of a pyroglobulin may be correlated with a submolecular immunoglobulin unit. That the pyroglobulin precipitate has biologic activity similar to that of aggregated normal immunoglobulin G(2) is of additional interest. The possibility that myeloma proteins are not abnormal proteins but normal proteins produced in excess quantities must be considered in the case of such a myeloma protein as a pyroglobulin. Certain identification of the presence of a normal counterpart present in small amounts in normal serum has not yet been possible.

The pyroglobulin described has been shown to be a 7S type of immunoglobulin(2) with characteristics of a G myeloma protein. The thermoprecipitable characteristics of the pyro-

TABLE I. Comparison of Thermoprecipitability at 56°C of Whole Pyroglobulin, Normal F2 and Their H and L Preparations.

Test solution	O.D. at 280 mμ		
	Supernatant	Precipitate	% in precipitate
33.3% SAS fraction of myeloma serum	3.6	41	92
Normal F2	45.2	1.06	2
H chain myeloma serum	6.2	12.3	66
L " " "	1.13	.82*	42
H " F2	16.6	1.15	5.9
L " "	1.28	5.75*	81

* Soluble at 100°C.

globulins are different from those of Bence-Jones proteins since the pyroglobulin precipitate formed by heating to 56°C does not dissolve at 100°C, a characteristic of Bence-Jones proteins. This characteristic has been demonstrated also for L chains from normal F2 immunoglobulins(6) and this property was observed in both the F2 L and myeloma L chains separated in the present study.

Up to 10 bands of L chains have been observed in L chains prepared from normal immunoglobulin G when the chains were studied by starch gel electrophoresis at pH 7-8 with urea and glycine(5). An L chain preparation from an individual myeloma protein would have the migration of one of the 10 L chain bands(5). Such a result was observed with the L chain from the myeloma protein presently described.

We propose that the thermoprecipitable protein described be categorized as a subclass of myeloma immunoglobulin G, termed G myeloma P (for pyroglobulin) and that the thermoprecipitable H chain be termed γ (pyroglobulin) using presently recommended nomenclature(7).

Summary. A G myeloma pyroglobulin was reduced, alkylated and separated into "heavy" and "light" polypeptide chains. The type of thermoprecipitability which characterized the pyroglobulin was localized to the "heavy" chain. It is suggested that this myeloma protein is a subclass which could be termed G myeloma P (pyroglobulin).

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Use of Peritoneal Dialysis in Experimental Amphetamine Poisoning. (30511)

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The amphetamines are important therapeutic agents which are used primarily for suppression of appetite, reduction of fatigue, and central nervous system stimulation. Because these drugs also create a state of exhilaration and euphoria, they have been abused through non-medical channels. Considerable attention has been focused on their widespread and indiscriminate use(1). Although they are considered to be relatively nontoxic, serious clinical manifestations of toxicity have been associated with overdosage(2). A recent report(3) that death occurred as a result of methamphetamine poisoning, indicated that

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