A NEW CLASS OF HUMAN IMMUNOGLOBULINS

I. A Unique Myeloma Protein

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Three main classes of human serum immunoglobulins are currently recognized: the IgG (γ_2-globulins), IgA (γ_{1A}, β_{2A}-globulins) and IgM (γ_{1M}, β_{2M}, or 18S γ-globulins). In addition there are low molecular weight immunoglobulins, most readily found in the urine. Each of these normal immunoglobulins has counterparts associated with plasmacytic or lymphocytic neoplasms, *i.e.* G (γ_2) myeloma proteins, A (γ_{1A}-, β_{2A}-) myeloma proteins, and M (Waldenström's) macroglobulins of serum and Bence Jones proteins of the urine. An atypical myeloma protein which does not fall within this present classification of immunoglobulins, therefore, would be of particular interest because it might represent either a very abnormal protein or a new class of immunoglobulin. Such a unique myeloma protein, found in the serum of patient S. J. with histologically proven multiple myeloma, is the subject of the present report.

Several observations had previously indicated that the myeloma protein in patient S. J. might be atypical. These observations took two forms. Papain treatment of human G myeloma proteins revealed that most proteins of this class yield fast Fc fragments of uniform electrophoretic mobility, but the Fc fragments of S. J. myeloma protein migrated more rapidly than typical Fc fragments (1). Secondly, studies of the turnover of normal IgG in patients with multiple myeloma revealed a uniform effect of G myeloma proteins in accelerating IgG catabolism, except in the case of S. J. and another patient (2). This was evidence that the S. J. protein differed from the typical G myeloma proteins and from the bulk of normal IgG in terms of the structures effecting IgG catabolism.

On the basis of these indications that the S. J. myeloma protein had unusual properties, detailed immunochemical and physicochemical studies were undertaken. The results show that the S. J. myeloma protein possesses a light polypeptide chain structure similar to that of other serum immunoglobulins but is unique in properties of the heavy polypeptide chains.

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Materials and Methods

The myeloma serum was obtained from S. J., a white male aged 57. At the time when serum was obtained for study, he had a 3 month history of low back pain. Bone marrow aspiration showed sheets of immature plasma cells, Bence Jones protein was present in the urine, and there was generalized osteoporosis, with collapse of the second lumbar vertebra. Subsequently, extensive osteolytic lesions typical of myeloma were observed. Autopsy 14 months from the time of serum study showed widespread and poorly differentiated plasma cell myeloma.

Quantitative paper electrophoresis, starch gel electrophoresis, immunoelectrophoresis, Ouchterlony tests, and analytical ultracentrifugation were carried out according to standard procedures (3). The myeloma protein was isolated from the serum by block electrophoresis, followed by column chromatography on DEAE cellulose as described for the isolation of A myeloma proteins (3). Preparation of normal IgG and IgM has been described (3) and IgA was generously made available by Dr. J. P. Vaerman and Dr. H. Fudenberg.

Papain digestion was by the method of Porter (4), restricting the time of digestion to 1 hour. Reduction and alkylation were by a modification of the method of Fleischman, Pain, and Porter (5). The myeloma protein (20 mg/ml in 0.5 M pH 8.0 tris HCl buffer) was treated with 0.1 M mercaptoethanol for 1 hour at room temperature, and then cooled in an ice bath. An equal volume of 0.1 M iodoacetamide in the same buffer at 0°C was added, and the mixture maintained at 0°C for 1 hour. The mixture was then dialyzed against several changes of 100 volumes of distilled water at 4°C for 6 hours, then dialyzed against 200 volumes of 1 M acetic acid for 16 hours at 4°C.

Heavy and light chains were separated on a column formed of a mixture of sephadex G-100 (1 part by weight) and sephadex G-200 (2 parts) equilibrated with 1 M acetic acid as suggested by Small *et al.* (6). Four ml of solution containing 40 mg of reduced and alkylated protein was applied to a column 79 x 1.8 cm. The eluate was collected in 4 ml fractions. The separation was carried out at 4°C.

Rabbit antiserums to IgG, IgA, and IgM were obtained by methods already described (3). Antiserum to the S. J. myeloma protein was obtained from two rabbits immunized with the isolated myeloma protein. Five and four-tenths mg protein in 2 ml saline emulsified with 2 ml Freund's complete adjuvant were injected intramuscularly in two sites, and the injections repeated after 4 weeks. After 6 weeks, the animals received a course of 5 injections on alternate days containing a total of 3 mg of alum-precipitated protein. The animals were bled 6 days after the end of the course. These antiserums were made specific for the S. J. myeloma protein by two methods. Absorption with a type L (II) G myeloma protein removed antibodies to type L (II) light chain determinants and to IgG, and was followed by absorption with serum from a case of hypogammaglobulinemia to remove small amounts of antibody to α- and β-globulins. Alternatively the antiserum was absorbed with 1/10 volume of normal human serum. Although most human serums contain the related class of immunoglobulin, the concentration of this component is insufficient to reduce materially the amount of specific antibody by this absorption (7).

RESULTS

S. J. Serum and Myeloma Protein.—Total serum protein concentration was 8.0 gm/100 ml. Paper electrophoresis (Fig. 1) showed an abnormal peak of protein migrating in the slower portion of the β-globulin region. The concentration of the anomalous protein was 3.9 gm/100 ml. Starch gel electrophoresis (Fig. 1) similarly showed a discrete abnormal protein band, which readily penetrated the gel and had a mobility consistent with a β-globulin.

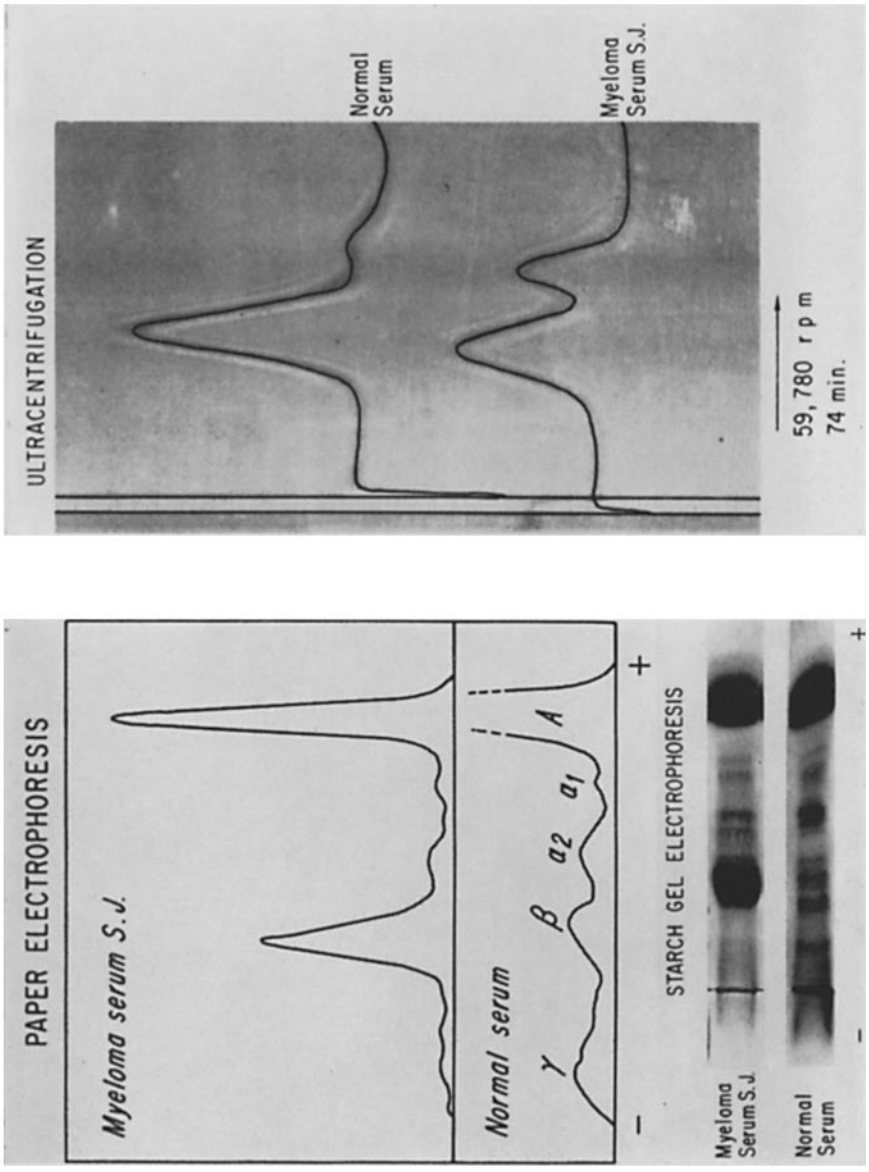

FIG. 1. Demonstration of the myeloma protein in the serum of patient S. J. On filter paper electrophoresis the protein migrates as a single peak in the β-globulin region. Starch gel electrophoresis demonstrates the protein as a single intense band. Ultracentrifugation shows the 7S component of S. J. serum to be increased compared with that of normal serum.

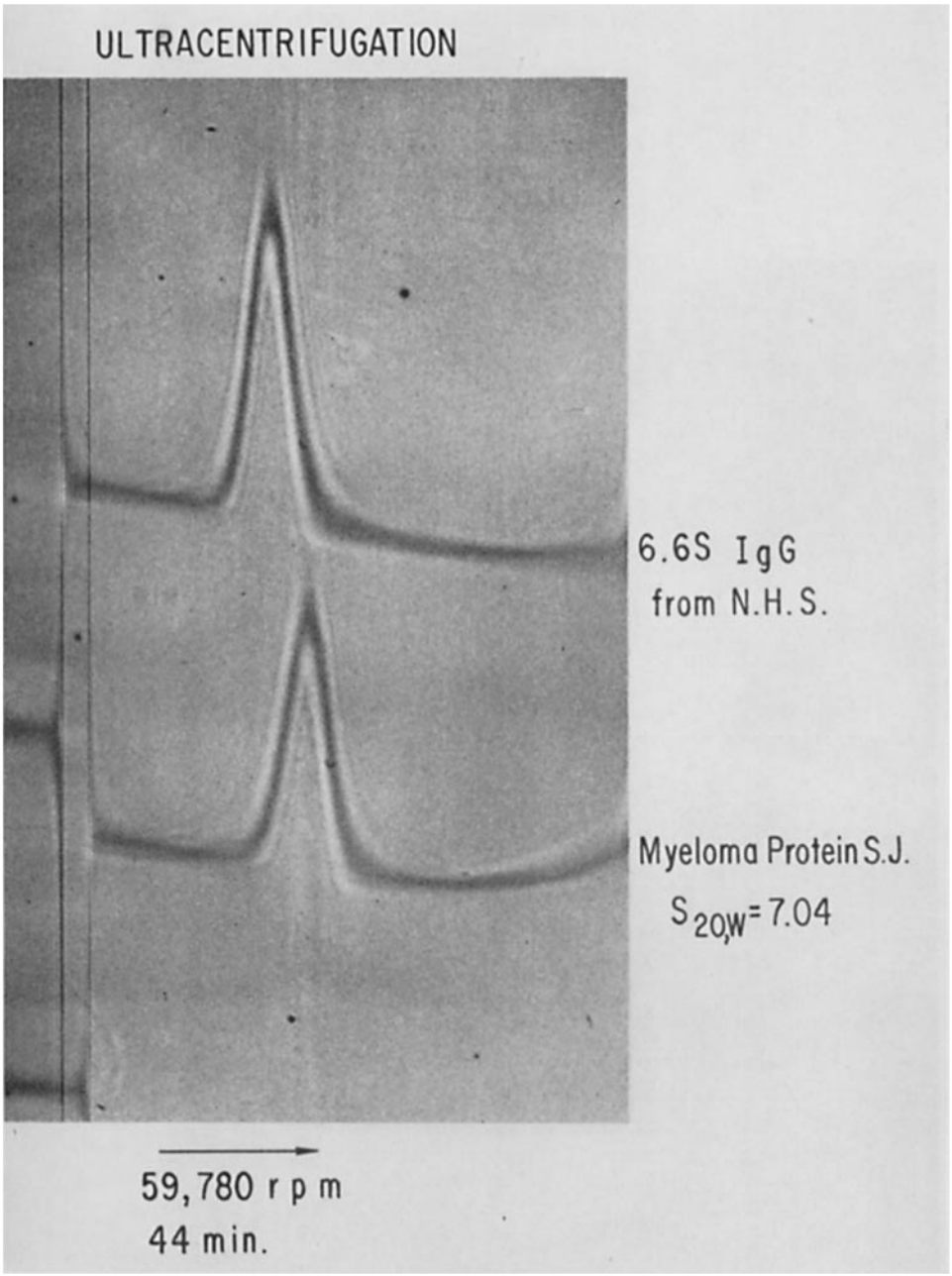

FIG. 2. Ultracentrifugal analysis of the isolated S. J. myeloma protein, compared with IgG from normal serum. Analysis was carried out at 59,780 RPM in a Spinco model E ultracentrifuge. Photograph obtained 44 minutes after reaching full speed. The S. J. myeloma protein sediments as a symmetrical peak of 7.04S. Protein concentration was approximately 5 mg/ml in 0.14 M NaCl. *N. H. S.*, normal human serum.

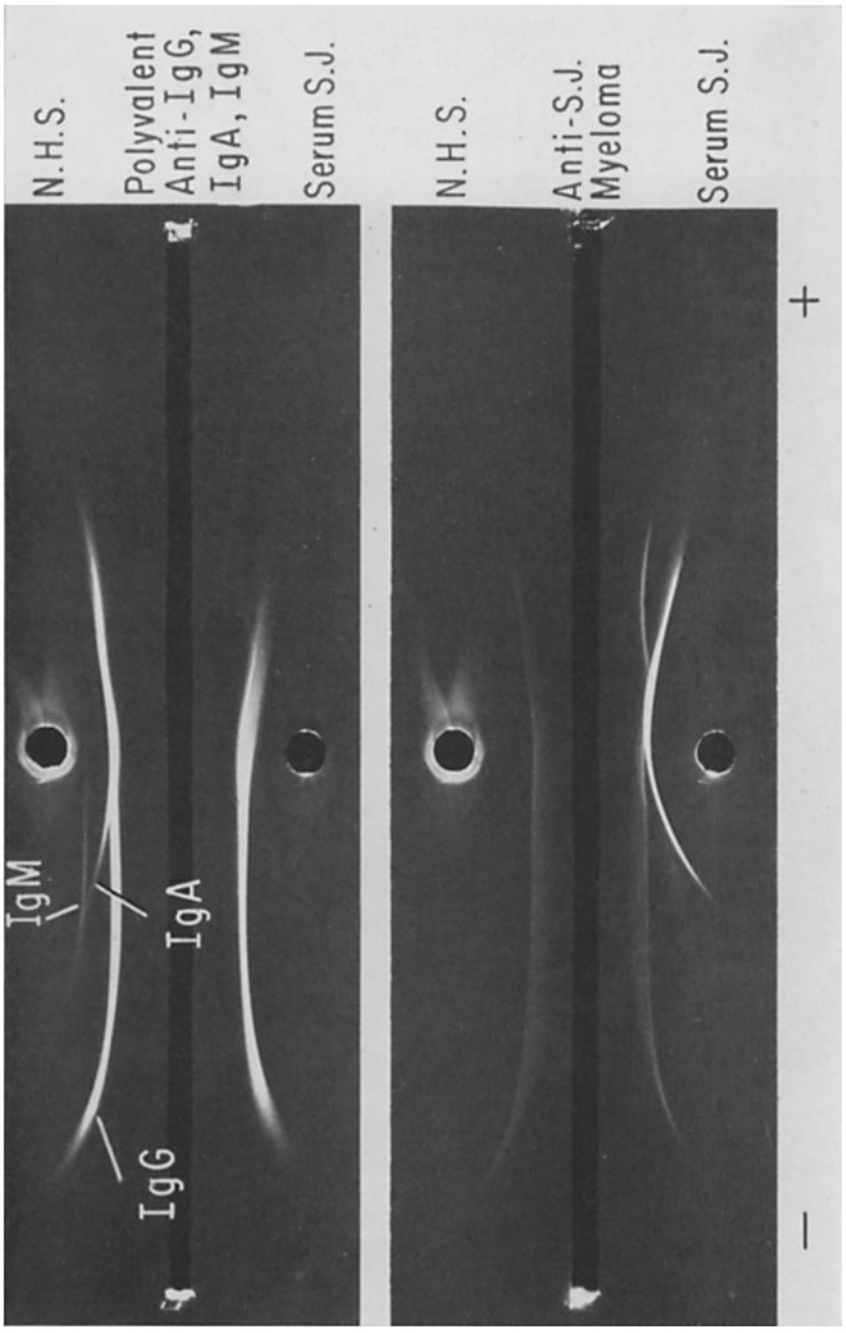

FIG. 3. Immunoelectrophoretic comparison of S. J. myeloma serum with normal serum. Polyvalent antiserum reacting with IgG, IgA, and IgM was used in the upper analysis. Antiserum to the isolated S. J. myeloma protein was used in the lower analysis. *N. H. S.*, normal human serum.

Ultracentrifugation of S. J. serum (Fig. 1) showed an elevated 7S peak, comprising 33 per cent of the total protein. The isolated protein sedimented as a symmetrical peak with a sedimentation coefficient ($S_{20,w}$) of 7.04S at a protein concentration of 0.5 gm/100 ml (Fig. 2).

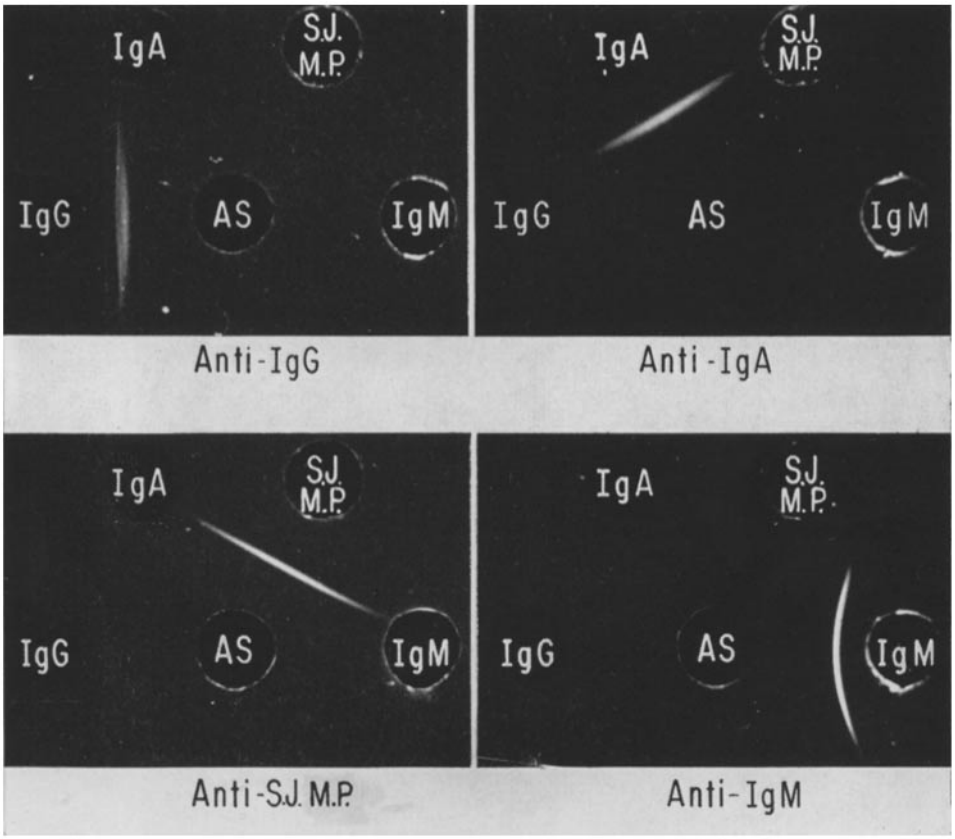

FIG. 4. Ouchterlony analyses showing the antigenic distinctiveness of S. J. myeloma protein. Antiserums specific to IgG, IgA, and IgM fail to react with S. J. myeloma protein, but do react with proteins of appropriate class isolated from normal serum. Antiserum specific to S. J. myeloma protein reacts with this protein, but not with IgG, IgA, or IgM. Protein concentration 0.5 mg/ml. The antiserum (*AS*) is indicated below each test. *M. P.*, myeloma protein.

Immunoelectrophoretic studies of the whole serum and isolated protein were carried out with polyvalent and specific antiserums. Immunoelectrophoretic analyses using an antiserum reactive with IgG, IgA, and IgM revealed only one precipitin line which was slightly broadened toward the anodal end

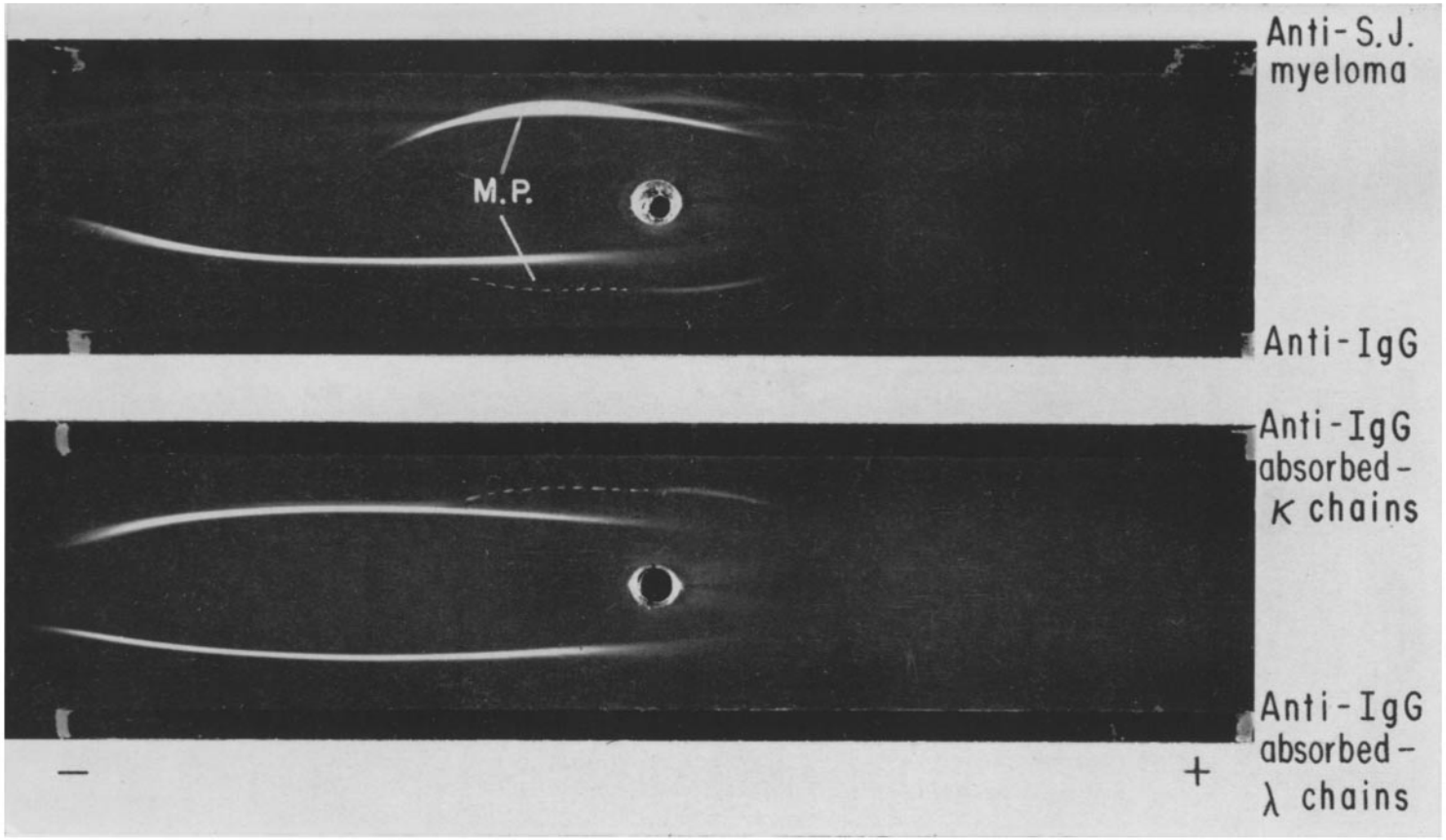

FIG. 5. Evidence for antigenic determinants of type L (II) in S. J. myeloma protein from immunoelectrophoresis of S. J. serum. Upper half: antiserum to IgG which reacts with specific IgG, type K (I) and type L (II) determinants reveals an IgG precipitin line and an additional line in the form of two arcs close to the antiserum trough. The more cathodal of these arcs is faint, and has been emphasized by the dashed line in the photograph. This arc is of similar mobility to the myeloma protein; the more anodal arc is of similar mobility to the serum Bence Jones protein. Lower half: Absorption of the antiserum with a type K (I) Bence Jones protein does not affect the precipitin lines. Absorption with type L (II) Bence Jones protein removed the ability of the antiserum to detect the myeloma protein and the Bence Jones protein. *M. P.*, myeloma protein.

(Fig. 3). Immunoelectrophoretic testing of S. J. serum against antiserums reacting specifically with IgG, IgA, or IgM failed to reveal any myeloma protein.

Antiserums prepared in rabbits to the isolated S. J. myeloma protein revealed the myeloma protein as a heavy precipitin arc in the β-globulin region of S. J. serum (Fig. 3). The antiserum to S. J. myeloma protein also produced a faint precipitin line with the IgG of S. J. serum and of normal serum (Fig. 3). Absorption of the antibodies reactive with IgG rendered the antiserum unreactive with IgG, IgA, and IgM (Fig. 4). The absorbed antiserum, however, was still reactive with the S. J. myeloma protein.

Direct comparison of the S. J. myeloma protein with representative IgG, IgA, and IgM preparations from normal serum (Fig. 4) confirmed the antigenic distinctiveness of the S. J. myeloma protein. The protein was not reactive with antiserums directed at the specific antigenic determinants of IgG, IgA, or IgM. Antiserums specific to the S. J. myeloma protein were unreactive with IgG, IgA, and IgM.

Further immunoelectrophoretic studies demonstrated that, in addition to distinctive antigenic properties, the S. J. myeloma protein shared some antigenic properties in common with the major immunoglobulin classes of normal serum. Immunoelectrophoretic analysis was carried out using an antiserum known to react with type K (I) and type L (II) light chain determinants. This antiserum revealed only the IgG arc in normal serum (not illustrated), but S. J. serum showed an extra line in the form of two arcs close to the antiserum trough (Fig. 5). The more cathodal of these arcs corresponded in mobility to the myeloma protein. Absorption of the antiserum by a type K (I) Bence Jones protein did not change the immunoelectrophoretic pattern, but absorption by a type L (II) Bence Jones protein, caused the extra line to disappear (Fig. 5, lower half). This was evidence that the serum S. J. myeloma protein possessed type L (II) antigenic determinants. The presence of type L (II) determinants on the S. J. myeloma protein was confirmed in studies of isolated light polypeptide chains (see below). This finding emphasized the structural relationship between S. J. myeloma protein and the immunoglobulin family.

The immunoelectrophoretic studies revealed an additional anomalous protein which migrated on the anodal side of the myeloma protein (Figs. 3 and 5). The precipitin line of this protein fused with the precipitin line of the S. J. myeloma protein (Figs. 3 and 5). This component was identified as a Bence Jones protein and was shown to have the same electrophoretic mobility and antigenic determinants, type L (II), as the Bence Jones protein present in the urine of this patient.

Substructure of the S. J. Myeloma Protein.—The composition of S. J. myeloma protein was investigated by reduction and alkylation to obtain the constituent polypeptide chains, and by papain digestion. The molecular subunits were compared with the components of IgG obtained by the same technics.

Reduction and alkylation caused dissociation of the constituent polypeptide chains into two groups, designated heavy and light on the basis of properties described below. These subunits were separated by filtration through a sephadex column in 1.0 M acetic acid (Fig. 6) or by starch gel electrophoresis with acid urea buffer in the manner of Poulik and Edelman (8). The last eluted peak from the sephadex column, tubes 42 to 55 in Fig. 6, contained 23 per cent of

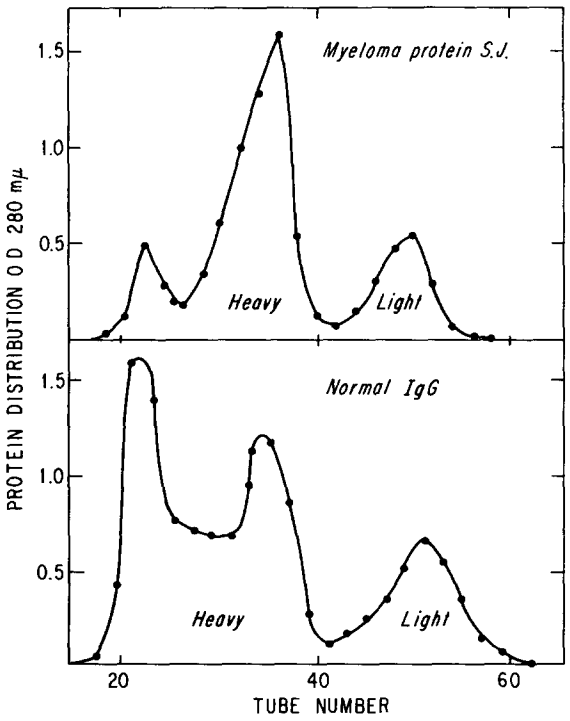

FIG. 6. Comparison of the products of reduction and alkylation of S. J. myeloma protein and of normal IgG. Gel filtration was performed on the same sephadex column in 1 M acetic acid. Heavy chains from both proteins form 77 per cent of the total eluted material.

the total protein. The proteins in this peak had starch gel electrophoretic characteristics of L polypeptide chains. Immunochemical tests using specific antiserums,—reacting specifically with S. J. myeloma protein or with type K (I) or type L (II) antigenic determinants,—revealed that the polypeptide chains in this last peak contained only type L (II) antigenic determinants, also indicating that the light polypeptide chains were in this column fraction.

The first eluted small peak and the first major protein peak, tubes 20 to 40 in Fig. 6, contained the heavy polypeptide chains. These fractions comprised 77 per cent of the total protein. The heavy chains of S. J. myeloma protein migrated less far than the light chains on starch gel electrophoresis in acid urea

and, indeed, migrated more slowly than heavy chains from G myeloma proteins. Heavy chain fractions did not precipitate on Ouchterlony analysis with specific antiserum to S. J. myeloma protein, but they did inhibit the ability of the antiserum to precipitate the intact S. J. myeloma protein. Thus, antigenic determinants characteristic of the S. J. myeloma protein were present in the heavy polypeptide chains.

In sum, these observations on the subunits obtained by reduction and alkylation indicate that the S. J. myeloma protein is composed of heavy chains of an unusual and distinctive antigenic type and of light chains of type L (II).

The subunits from S. J. myeloma protein and normal serum IgG obtained by reduction and alkylation (Fig. 6) were compared. The heavy polypeptide chains were eluted in tubes 20 to 40, and comprised 77 per cent of the total protein in both cases. These fractions also contained distinguishing specific antigenic determinants. The light polypeptide chains were similarly comparable, being eluted in tubes 42 to 55 and in having light chain–specific determinants, both types K (I) and L (II) in the case of normal IgG and only type L (II) in the case of the S. J. myeloma protein.

Several characteristics of the sephadex fractions from reduced and alkylated S. J. myeloma protein deserve additional comment. (*a*) No fractions contained material precipitable by antiserum specific for IgG, IgA, or IgM antigenic determinants. Thus the specific determinants of the three known classes of immunoglobulins were not detected in the S. J. myeloma protein even after reduction and alkylation procedures. (b) No fractions contained material precipitable by antiserum for type K (I) light chain antigenic determinants, indicating the absence of contamination of the S. J. myeloma protein preparation with normal immunoglobulins. (*c*) Light polypeptide chains isolated from the S. J. myeloma protein reacted much more strongly with antiserum against type L (II) antigenic determinants (anti–type L Bence Jones protein), than did the intact protein. This phenomenon is believed to be due to the exposure of light chain determinants which are "buried" in the intact molecule, and is discussed further in the accompanying paper (7).

Papain digestion of the S. J. myeloma protein yielded fragments sedimenting at 3.4S in the ultracentrifuge. Immunoelectrophoretic analysis, using antiserum to the myeloma protein, revealed two antigenically and electrophoretically distinct fragments (Fig. 7). After separation by chromatography on DEAE cellulose both fragments sedimented at approximately 3.7S. In these respects the fragments were similar to the Fab (slow) and Fc (fast) fragments of IgG. The Fc fragments of the S. J. myeloma protein, however, migrated more rapidly toward the anode than did the Fc fragments of IgG (Fig. 7). In this respect the Fc fragments of S. J. myeloma protein also differed from the Fc fragments of the majority of G myeloma proteins (1, 9, 10). The unusual

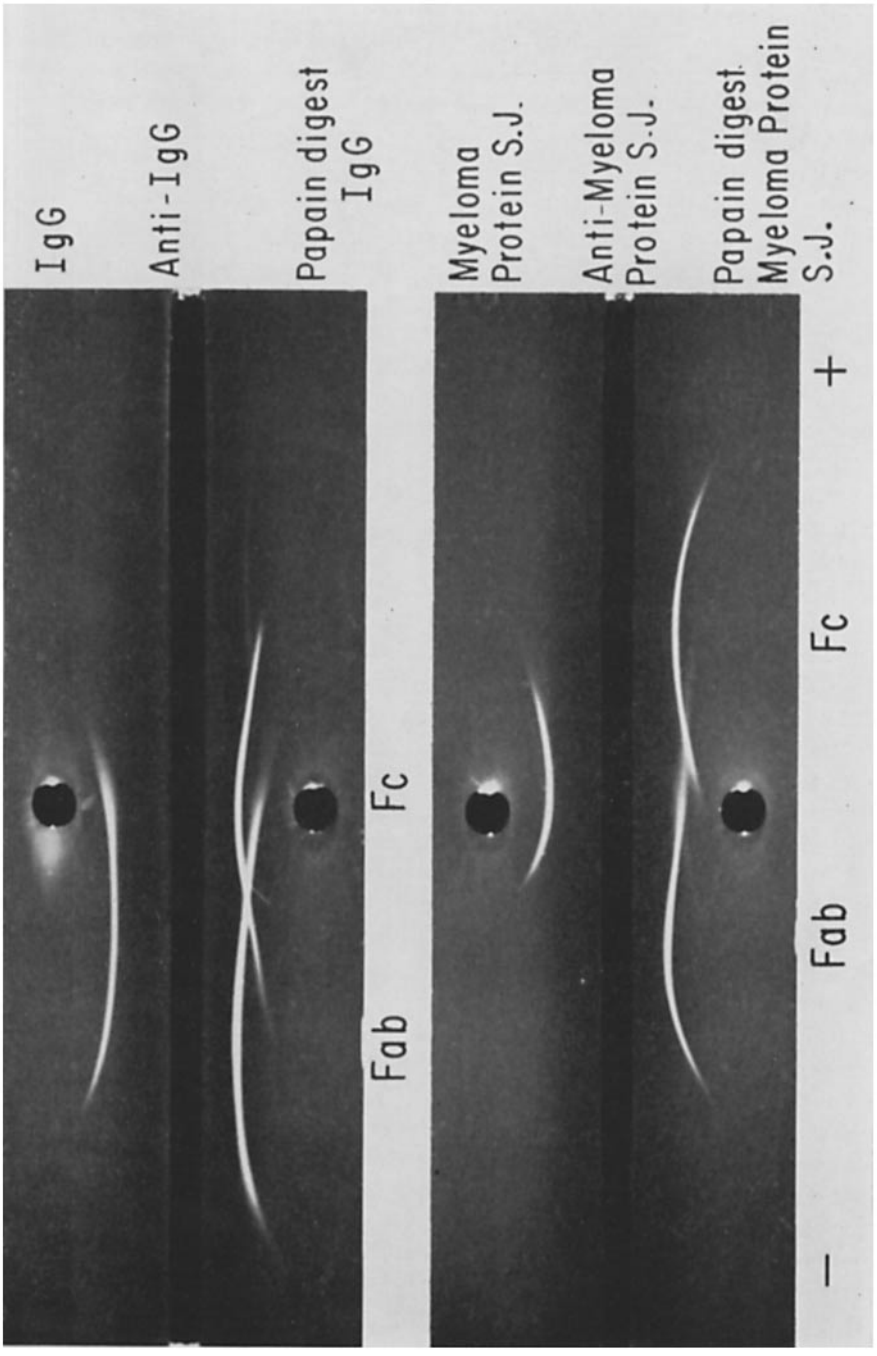

FIG. 7. Comparison of papain digests of IgG (upper half) and S. J. myeloma protein (lower half). Immunoelectrophoretic analysis of the digested myeloma protein using its own antiserum reveals two antigenically and electrophoretically distinct fragments, Fab (slow) and Fc (fast), as well as some residual undigested protein. The mobility of the Fab fragment is within the range of Fab fragments of normal IgG, but the Fc fragment migrates more rapidly than do the Fc fragments of normal IgG.

mobility of the Fc fragment provides further evidence of distinctive properties of the heavy chain of S. J. myeloma protein.

DISCUSSION

Myeloma proteins comprise a group of pathological globulins which are identified by their special antigenic and structural relationships to the immunoglobulins of normal plasma, and by their origin in neoplastic plasma cells. The anomalous protein of S. J. serum had a dual relationship to other myeloma proteins, *i.e.*, it was similar to typical myeloma proteins in many respects, but it also had unique properties. Features characteristic of all myeloma proteins included heavy and light polypeptide chain composition. The light chains had typical type L (II) antigenic determinants. Papain treatment produced Fab and Fc fragments in the same ratio as for IgG. A Bence Jones protein of the same type (type L) was present in serum and in urine. Finally, sheets of plasma cells found on marrow biopsy are presumed to be the site of myeloma protein synthesis.

Three features of the S. J. myeloma protein helped to distinguish it from G myeloma proteins, A myeloma proteins, or M macroglobulins of Waldenström. The first of these was immunochemical; *i.e.*, the absence of specific IgG, IgA, or IgM determinants. The second feature was the unusually rapid electrophoretic mobility of the Fc fragment obtained by papain digestion. Most, but not all, G myeloma proteins yield Fc fragments of slower mobility (1, 11). The third was the lack of effect of this myeloma protein on the rate of catabolism of normal IgG (2). All three of the atypical features of the S. J. myeloma protein reflect specific properties of the heavy polypeptide chains.

An unusual protein of this type might possibly arise from a major disorder of the protein synthetic mechanism within the malignant plasma cells with the result that a completely abnormal heavy polypeptide chain was formed. Alternatively, the S. J. myeloma protein might represent the quantitative increase of a previously unrecognized normal class of immunoglobulins.

A number of observations indicated that the heavy chains were normal in many features, *i.e.*, they combined with light chains in a similar ratio as do those in IgG and other immunoglobulins (12–14); they were of approximately the same size as γ-heavy chains, as seen by their behavior on sephadex column filtration and by their presence in a 7S molecule; and papain hydrolyzed the S. J. myeloma protein in a manner similar to its action on IgG. Finally, and most persuasively, an antigenically related protein in low concentration has been demonstrated in many normal serums as described in the following paper (7). These observations indicate that the S. J. myeloma protein does not represent a gross abnormality of heavy chain synthesis by the neoplastic cells. Apparently the S. J. plasmacytoma was unusual in its production of a myeloma protein representative of a class of immunoglobulins which form only a small proportion of the total immunoglobulin population of normal serum.

These observations do not constitute proof that the S. J. myeloma protein is normal in every respect. Myeloma proteins may have individually specific antigenic determinants (15). The S. J. myeloma protein may have such determinants, but these do not account for all of the immunochemical uniqueness of this myeloma protein. In particular, antigenic individuality of the Fc fragment obtained by papain digestion probably represents class-specific antigenic determinants, since the Fc fragment contains the class-specific antigenic character of IgG in man (16) and IgG and IgA of the mouse (17).

A survey of available myeloma serums was undertaken to determine the frequency with which the S. J. type myeloma proteins appeared. Of 91 additional myeloma proteins tested, none have been found to be specifically related to the S. J. myeloma protein. This indicates that the S. J. myeloma protein represents an uncommon class of myeloma protein.

SUMMARY

The unique myeloma protein from S. J., a patient with multiple myeloma, was isolated and characterized. It resembled other myeloma proteins in many respects. The S. J. myeloma protein migrated in a distinct peak in the slow β-globulin region on zone electrophoresis, appeared as a single band on starch gel electrophoresis, and sedimented at 7.04S in the ultracentrifuge. Papain and cysteine treatment produced Fc (fast) and Fab (slow) fragments. Reduction and alkylation of the myeloma protein produced heavy and light chains in a ratio of approximately 3:1. The S. J. myeloma protein had type L (type II) light chains. These were antigenically similar to the Bence Jones protein also found in this patient.

The S. J. myeloma protein was unique in the properties of its heavy chains. The myeloma protein (and its heavy chains and Fc pieces) did not contain antigenic determinants specific for IgG, IgA, or IgM. The myeloma protein (and its heavy chains), however, did contain antigenic determinants which are characteristic of a new class of immunoglobulin. The S. J. myeloma protein was unusual also in its effect on the metabolism of normal IgG and in the electrophoretic mobility of the Fc fragment produced by papain digestion.

No evidence was obtained to indicate that the entire heavy polypeptide of the S. J. protein was a grossly abnormal product of malignant cell metabolism. The unique properties of the S. J. myeloma protein (and its heavy chains) are believed to represent, in large measure, properties to be found in a small part of the normal immunoglobulin population.

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