STRUCTURAL STUDIES OF HUMAN 7S γ-GLOBULIN (G IMMUNOGLOBULIN)

FURTHER OBSERVATIONS OF A NATURALLY OCCURRING PROTEIN RELATED TO THE CRYSTALLIZABLE (FAST) FRAGMENT*

BY E. C. FRANKLIN,‡ M.D.

(From the Department of Medicine and the Rheumatic Diseases Study Group, New York University School of Medicine, New York)

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The immunoglobulins in man consist of three major classes of proteins which are related to each other not only in function but also because of the existence of certain structural units common to all of them. Differences between them reflect the presence in each class of a unique structural unit, the heavy chain.[1]

Characterization of the normal immune globulins has been greatly aided by studies of several closely related proteins produced in large amounts in certain proliferative disorders of plasma cells and lymphocytes. Although these proteins differ from the heterogeneous population of immunoglobulins in some ways (1, 2), they resemble them sufficiently in chemical structure and in their genetic control to allow their use as models of each of these proteins. Their greater homogeneity has led to the suggestion that they may be analogous to individual purified antibodies (3). The paraproteins are particularly useful in those instances where they are related to some of the struc-

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[1] A number of laboratories have independently carried out studies on these fractions, and consequently different names have been used for products which appear to be similar. In an effort to eliminate the resulting ambiguities a committee on nomenclature of the World Health Organization (Bull. 447) World Health Organ. 1964, 30, has recently recommended the following nomenclature which will be used in this report:

Classes of immunoglobulins: G Immunoglobulin (IgG) for 7S (γ_2)-globulin; A Immunoglobulin (IgA) for γ_{1A}-globulin; M Immunoglobulin (IgM) for γ_{1M}-globulin.

Polypeptide chains: heavy and light for the major types although the existence of a third type remains a distinct possibility. γ-, α-, and μ-chains denote the heavy chains of IgG, IgA, and IgM respectively.

Fragments produced by papain: Antigen-binding fragment (Fab) will replace slow (AC) fragment. Crystallizable fragment (Fc) will be used instead of fast (B) fragment. Fragment d (Fd) will replace A piece.

In most instances there is a close correspondence between the old and new terminologies. Only in the case of the papain fragments is there a direct contradiction between the terms previously employed by us (Fragments AC, and B) and the new proposed terminology (Fab, and Fc) respectively.

tural units of the immunoglobulins obtainable only after reductive cleavage, a chemical procedure which may alter some of their chemical and antigenic properties. For example, studies of Bence Jones proteins (BJP), which generally are dimers of light chains, have provided much information about the structure of light chains (4). With the discovery of five patients with heavy chain disease and the ready isolation of naturally occurring proteins closely related to the crystalline (Fc) fragment of G immunoglobulin from their sera and urines, it has become possible to use these proteins to extend previous studies obtained with artificially produced heavy chains, and to define their precise relation to the products of papain and pepsin digestion of IgG. The clinical picture of these patients and some preliminary chemical studies of the proteins have been published (5–7), and these will be summarized only briefly for purposes of orientation. Turnover studies with I^{131} G immunoglobulin clearly demonstrated that the protein was not a breakdown product of IgG and led to the conclusion that it was independently synthesized. Both the serum and urine proteins had identical electrophoretic mobilities on starch and paper electrophoresis, intermediate between the γ- and β-globulin peaks. On starch gel electrophoresis, four distinct bands were visible. The serum and urine proteins had sedimentation coefficients of 3.8S. Antigenic studies showed a reaction of complete identity with the non-antibody (Fc) fragment of IgG, partial identity with IgG, and complete non-identity with the IgA and IgM fractions, the antigen-binding (Fab) fragment from IgG and normal and pathologic L chain proteins. The protein was Inv (a−b−). Initially, it was only Gm (e+), but later it also had Gm (b) activity. As a result of these studies, the protein was shown to be closely related to the crystallizable (Fc) fragment. Since this is the part of the molecule that is responsible for most of the properties currently ascribed to the heavy chain, the disorder was called heavy chain disease by Osserman (5 a). In view of the uncertainty as to both the precise nature and number of chains present in G immunoglobulin, it seems best at this time to retain this name. However, it must be emphasized that this protein consists primarily, if not entirely, of the part of the heavy chain found in the Fc papain fragment. The synthesis of this type of fragment in this patient suggests that this may be a natural structural unit of IgG. If this were the case, the disorder should be called accordingly.

Materials and Methods

Techniques for Isolation.—Protein was isolated free of all contaminants except for traces of transferrin by starch zone electrophoresis or electrophoresis on polyvinyl chloride (8).

Preparative Procedures.—Reduction and alkylation were done either using mercaptoethanol in 8 M urea as described by Edelman and Poulik (9); or by the technique of Fleischman, Pain, and Porter in tris buffer pH 8.2 followed by 1 normal acetic acid (10).

Papain and pepsin digestions were performed as described previously, and the antigen-binding (Fab) and crystalline (Fc) fragments were separated by chromatography on DEAE cellulose (11).

Antisera were prepared in rabbits by the repeated injection into the foot-pads or the subcutaneous tissue on the back of the following antigens incorporated in an equal volume of Freund's adjuvant: (*a*) IgG (Lederle Laboratories, Pearl River, New York), purified by chromatography on DEAE cellulose, 6 rabbits; (*b*) Patient's serum or urine protein, 2 rabbits each; (*c*) Fab and Fc fragments of IgG, 1 rabbit each; (*d*) Papain and pepsin digests of patient's protein, 2 rabbits each.

Analytical Procedures.—(*a*) Immunoelectrophoresis and double diffusion in agar were performed by standard techniques as previously described (11). (*b*) Starch gel electrophoresis in pH 3.4 formate buffer in 6 M urea was performed as described by Edelman and Poulik (9). Starch gel electrophoresis without urea was performed at pH 8.2 in the discontinuous buffer system (12). (*c*) Sedimentation coefficients were determined in a Spinco model E ultracentrifuge with schlieren optical system, using double sector cells (13). (*d*) Molecular weights were performed by the Archibald technique of approach to equilibrium. Observations for each preparation were made on 6–12 frames taken at each of three different speeds so that a minimum of 18 points was used to calculate the value. Calculations were made as described by Trautman (13). (*e*) Fingerprinting: Two-dimensional descending chromatography (butanol acetic acid water, 4:1:5) for 21 hours followed by high voltage electrophoresis at pH 3.8 in a direction perpendicular to that of chromatography of performic acid–oxidized trypsin-digested material was performed as described by Katz, Dreyer, and Anfinsen (14) and has been described in detail in a previous publication (15). The peptide spots were stained with ninhydrin.

RESULTS

Studies of the Native Protein.—

Molecular weight: Table I lists the molecular weights determined by the Archibald technique of several preparations of serum and urine protein. They were the same and ranged from 49,000 to 55,000 with a mean of 51,000. The molecular weight was not changed significantly in 6 M urea or 1 N acetic acid. These values are similar to those reported by Edelman (9) and by Pain (16) for the heavy chains of IgG produced by reductive cleavage, and also similar to those of the Fc fragment produced by treatment with papain (11).

Electrophoresis in urea starch gel and in pH 8.2 starch gel: Fig. 1 *a* compares the electrophoretic migration in a urea gel of the protein to that of IgG, the Fc papain fragment, and heavy and light chains produced from G immunoglobulin by reductive cleavage. It can be seen that the mobility is different from that of artificially produced heavy and light chains and unlike that of the Fc fragment. Treatment with urea or acid did not alter this appearance; however, as will be discussed below, the mobility was changed by reductive cleavage. In a regular starch gel at pH 8.2, the banding characteristic of the protein was similar to that of the artifically produced Fc fragment, but the precise mobilities of the bands differed (Fig. 1 *b*).

Carbohydrate analyses were kindly performed by Dr. Z. Dische. Table II lists the carbohydrate composition of the serum and urine proteins. It is apparent that the protein contains large amounts of carbohydrate and that it is richer than G immunoglobulin or the Fc fragment prepared from IgG by papain (17, 18).

Immunologic studies: As previously reported, five antisera to G immunoglobulin demonstrated a reaction of complete identity between the patient's protein and the Fc fragment of IgG, and a reaction of non-identity with the Fab fragment. The results with several antisera to the serum and urine proteins varied. An early bleeding of three of the animals showed a distinct spur on the

precipitin line of the patient's protein extending beyond that of the crystallizable fragment of IgG. Absorption of these early antisera with purified Fc fragment left residual antibody, reactive with the proteins of this patient and with IgG. A large number of later attempts to repeat these studies with subsequent bleedings from the same rabbits failed to demonstrate this antigenic specificity and showed a reaction of complete identity between the patient's proteins and the Fc fragment. While these findings may be due entirely to the antigenic speci-

TABLE I

Molecular Weights and S Rates of Native and Reduced and Alkylated Proteins

Solvent	Native	Reduced and alkylated	Sedimentation coefficient*	
			Native	Reduced and alkylated
	mol wt	*mol wt*	*S*	*S*
0.15 M NaCl	53,000‡	—	3.8	—
	49,000	—	3.8	
	53,000	—		
	50,000	—		
6 M urea	50,000	29,000	1.51	0.76
	50,000	28,000	1.44	0.76
	49,000	26,000	1.50	0.84
		32,000		
		23,000		
		22,000		
8 M urea	49,000	24,000	0.92	0.56
4 M urea	55,000	22,000	2.52	1.00
Mean	51,000	26,000		

* As observed in the solvent.

‡ Serum. All others are urine protein.

ficity often seen in paraproteins (19–21), they raise the possibility that they may be due to the presence of certain determinants found in the antigen-binding (Fab) fragment. Studies with a single antiserum to IgG favor such a view (see below). However, the bulk of the evidence points against it. In particular, the failure of an antiserum to the Fab fragment to react with the patient's protein and conversely, the failure of three of four antisera to the patient's protein and two antisera to papain and pepsin digests to react with the Fab fragment argue strongly against any cross-reaction with the Fab fragment (Fig. 3 *c*). Other studies to be described below support these findings.

Studies of Structural Units of This Protein and Their Relation to G Immunoglobulin.—A number of degradative procedures previously used to study G im-

munoglobulins were employed to define the structure of this protein and its relation to the various well characterized products obtained by reduction and alkylation and by enzymatic degradation of IgG.

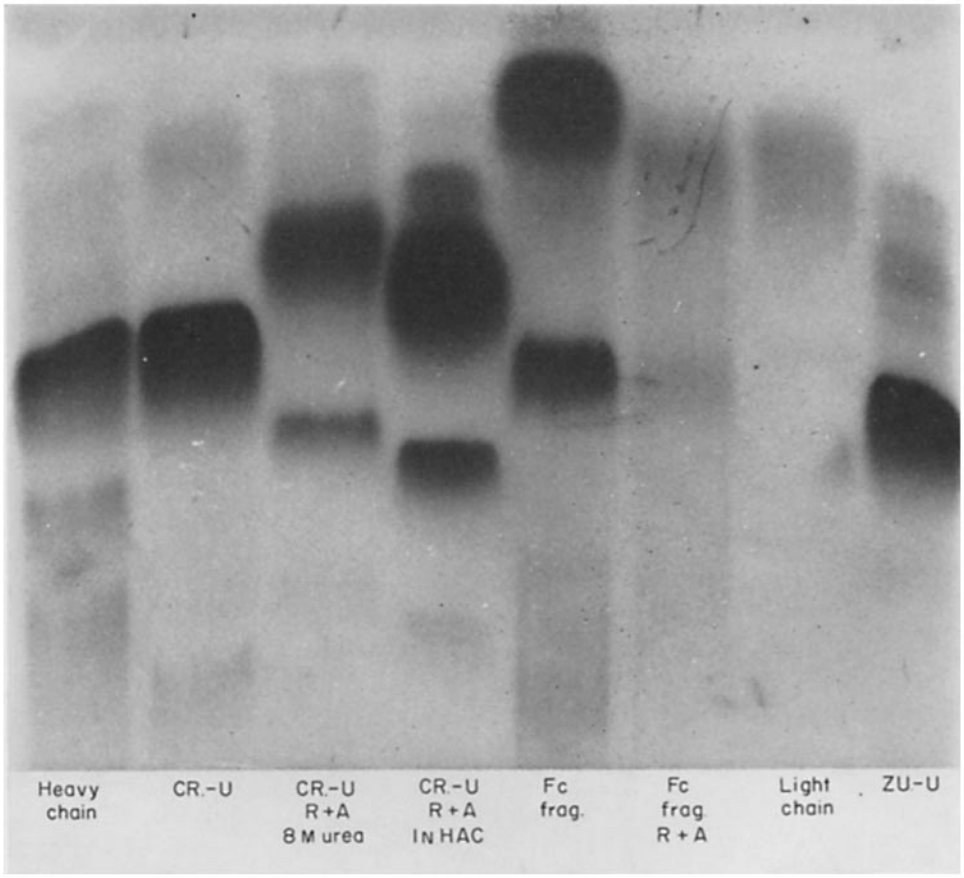


FIG. 1 *a*. Urea starch gel electrophoresis of heavy and light chains of IgG, Fab and Fc fragments, native and reduced, $(R + A)$, and the urine proteins of 2 subjects (CR. and Zu.) with heavy chain disease.

Reduction and alkylation were carried out in 8 M urea by the method of Edelman (9) or in pH 8.2 tris buffer followed by 1 N acetic acid (10). Mercaptoethanol was used in concentrations ranging from 0.025 M to 0.75 M. Most of the studies, except those where the antigenic properties of the products were examined, were carried out in urea.

Reduction and alkylation by the method of Edelman resulted in a significant

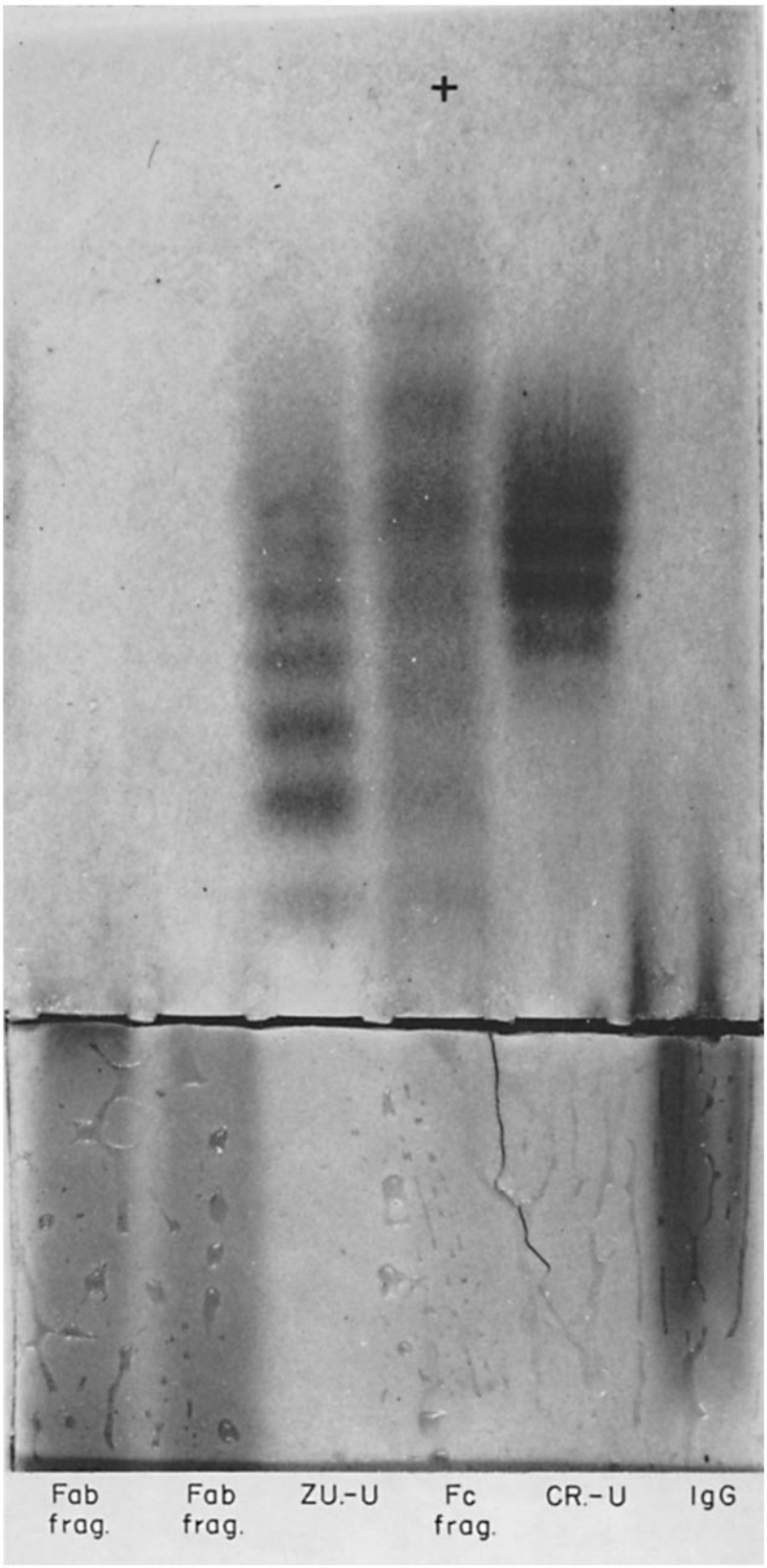


FIG. 1 *b*. Starch gel electrophoresis of IgG, Fab and Fc fragments of IgG, and the isolated urine proteins of 2 subjects with heavy chain disease. CR. is the patient in this study and reported in detail (6, 7); Zu. is Case No. 5 of Osserman (5 *b*).

fall in the sedimentation coefficient and a fall in molecular weight from an average of 51,000 to 26,000 (Table I). Starch gel electrophoresis in urea of reduced and alkylated preparations generally yielded a major broad band migrating slightly ahead of the native protein and a narrow one migrating more slowly (Figs. 1 *a* and 1 *b*). These bands did not have the same mobility as the Fc fragment reduced under similar conditions. In only one experiment, for as yet unexplained reasons, was an additional band seen. The two fragments produced by reduction and alkylation could not be resolved either on sephadex G 75 or 100 or by electrophoresis on a starch block or by immunoelectrophoresis, thus suggesting the possibility that two similar fragments were produced. A similar conclusion was independently arrived at by Dr. Frank Putnam on the basis of an amino acid analysis and fingerprint studies which showed the presence of only

TABLE II

Carbohydrate Analyses of Serum and Urine Proteins Compared to Those of G Immunoglobulin and Crystalline (Fc) Fragment

Protein	Hexose	Hexosamine	Fucose	Sialic acid
	per cent	*per cent*	*per cent*	*per cent*
Urine protein, CR	8	7	2	4
Serum protein, CR	8	7	2	4
IgG*	1.2	1.1	0.3	0.2
Fc Fragment‡	2.0	1.5	0.5	0.1

* Data from reference 17.

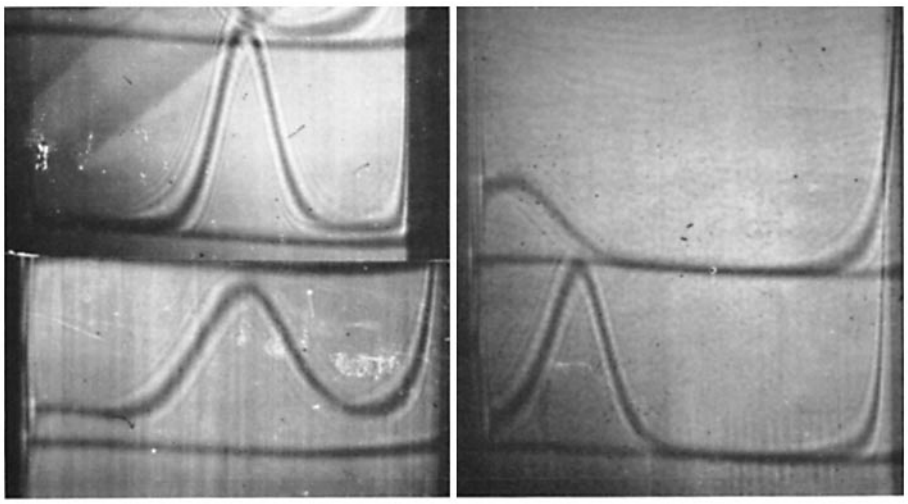
‡ Data from reference 18. Expressed as mg/gm starting protein. Total recovery 50 to 60 per cent.

25 peptide spots, approximately one-half the number expected on the basis of arginine and lysine content (22).

Enzymatic degradation with papain and pepsin was carried out in a manner similar to that previously employed for IgG. It is now accepted that papain splits IgG into two similar antigen-binding (Fab) fragments each carrying one light chain and a poorly characterized piece which may represent either a piece of heavy chain or a separate structural unit bound to the Fc fragment by an unusual type of chemical bond. The remainder of what is currently called the heavy chain is found in the Fc fragment. With certain antisera, a third (F′) component, antigenically related to the Fc fragment, can be seen (23). Pepsin destroys the Fc fragment, but leaves a piece closely related to the Fab fragment, the 5S component $(Fab')_2$ fragment, which can then be cleaved by a sulfhydryl reagent (24).

Papain digestion of the patient's protein yielded two major ultracentrifugal and immunologically identifiable components. The 3.8S component was changed into a much broader major peak with a sedimentation coefficient of about 3.5S,

making up 60 to 65 per cent of the total and a heterogeneous slowly sedimenting peak, making up 20 to 25 per cent of the total while about 10 per cent appeared as dializable peptides. (Fig. 2 *a*). The sedimentation coefficient of the slowly sedimenting peak could not be readily determined. Pepsin digestion (Fig. 2 *b*) resulted in the recovery of only about 30 to 40 per cent of the protein as a heterogeneous non-dializable component with a sedimentation coefficient of less



a *b*

FIGS. 2 *a* and 2 *b*. Ultracentrifugal patterns. Fig. 2 *a*. Native protein (top) and papain digest(bottom). Photos taken after 192 minutes, 52,640 RPM. Fig. 2 *b*. Pepsin digest of urine protein (CR.) (top) and native protein (bottom). Photos taken after 128 minutes, 52,640 RPM.

than 1. Similar studies of the pepsin-digested Fc fragment also yielded 20 to 30 per cent non-dializable material with properties similar to the pepsin digest of the patient's protein.

Immunologic studies comparing these fragments to the native protein and various structural units of IgG proved particularly useful in defining its precise structure and its relationship to the major fragments of IgG (25). The papain digest gave two lines which appeared to contain all the antigenic groups present in the native protein when tested with six antisera to IgG by the Ouchterlony technique. Pepsin digestion yielded a product that failed to react with two of five antisera to IgG and with one of four antisera to the patient's protein. As shown in Fig. 3 *a*, the pepsin digests gave a reaction of partial identity with the native protein and the Fc fragment, both of which formed marked spurs extending over the line formed by the pepsin digest. All of the antisera showed a reaction of

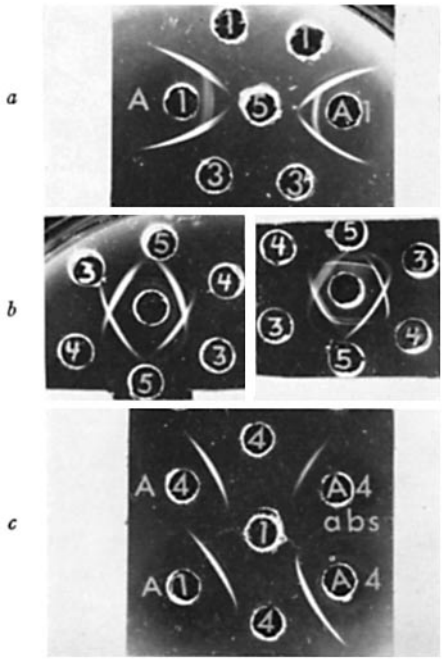


FIG. 3. *a*. Ouchterlony analysis with two antisera to the patient's urine protein (*A1*) in the center wells and *1*, urine protein CR.; *3*, Fc fragment; and *5*, pepsin digest of urine protein CR.

FIG. 3 *b*. Ouchterlony plates comparing two antisera to IgG in the central wells with: *3*, Fc fragment; *4*, Fab fragment; *5*, pepsin digest of urine protein (CR.). Five other antisera were similar to the antiserum on the left.

FIG. 3 *c*. Antisera to the patient's protein (*A1*) and to the Fab fragment (*A4*) and to the Fab fragment absorbed with light chains (*A4 abs*). The antigens are urine protein CR. (*1*) and Fab fragment (*4*). It can be seen that the antisera react only with the antigens used for immunization and not with the other antigens.

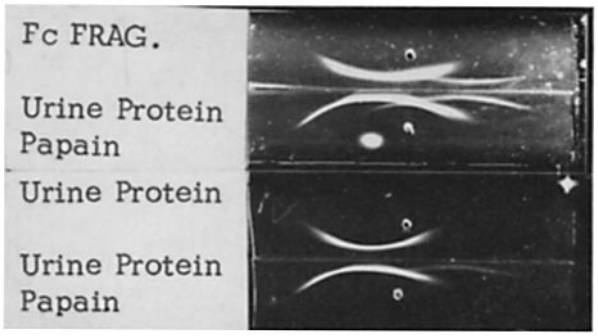


FIG. 4. Immunoelectrophoresis using an antiserum to the patient's urine protein in the center. Antigens: Fc fragment; urine protein (CR.), and urine protein CR. after papain digestion.

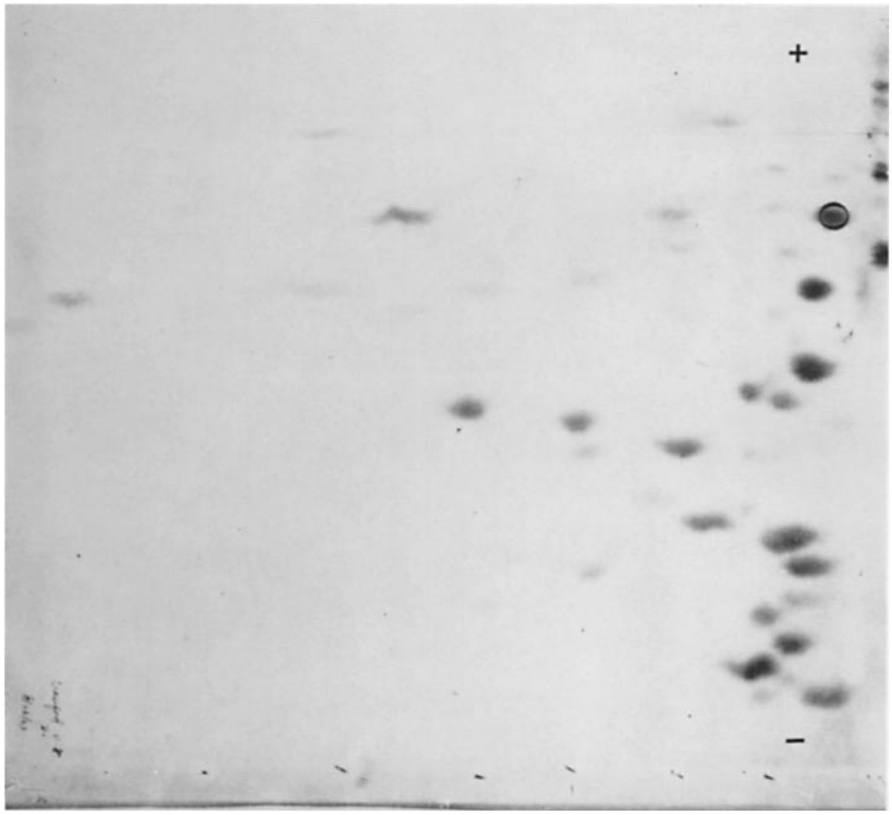


FIG. 5 *a*

FIG. 5 *b*

complete non-identity between the pepsin digest and artifically prepared light chains of G immunoglobulin. When compared to the Fab fragment of IgG, five of the antisera to G immunoglobulin also showed a reaction of non-identity. However, on numerous occasions, one antiserum showed a reaction of partial identity between the Fab fragment prepared from IgG and one of the lines formed by the pepsin digest (Fig. 3 *b*). It is possible that this is due to the presence of traces of contaminants present in these preparations. Of particular interest are the studies in Fig. 3 *c* with antisera to the patient's protein and to the Fab fragment. The former reacted only with Fc fragments and the patient's protein and failed to give a precipitin line with the Fab fragment. The antiserum

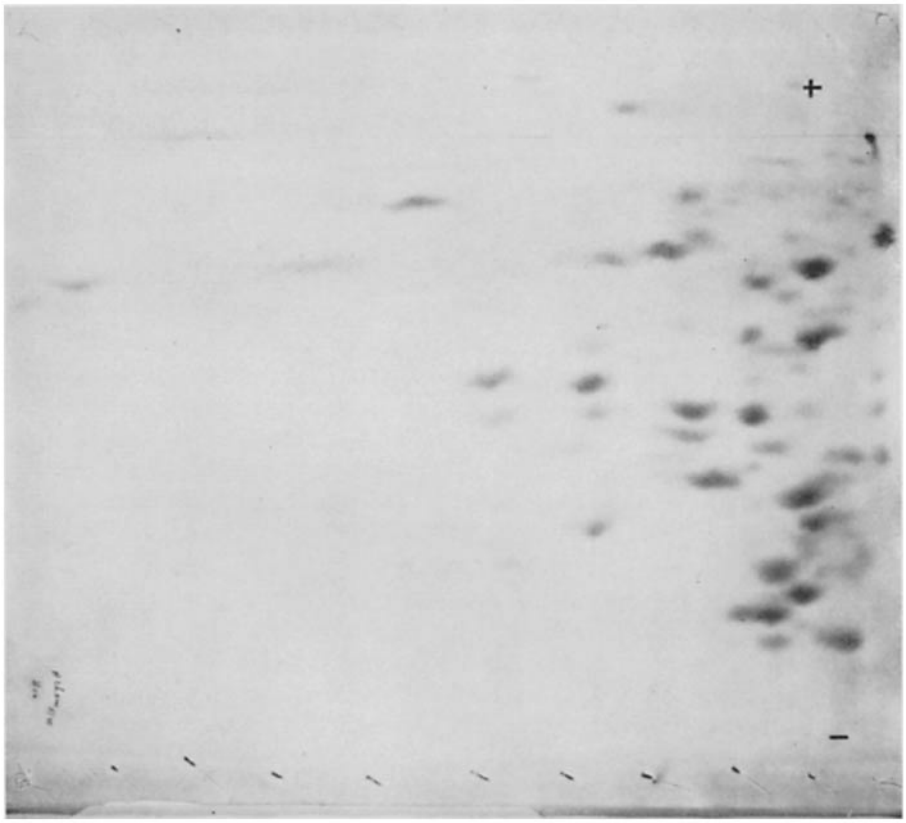


FIG. 5 *c*

FIGS. 5 *a* to 5 *c*. Fingerprint maps of: (*a*) CR. urine protein; (*b*) normal Fc fragment; (*c*) normal heavy chain. Chromatography in horizontal and electrophoresis in vertical direction. The spot marked by the circle is unique to the patient's protein.

to the Fab fragment either as such, or absorbed with light chains, reacted only with the Fab fragment but not with the patient's protein or the Fc fragment. While these studies suggested that the patient's protein consisted primarily of the Fc fragment and contained few, if any, of the Fab fragment determinants including those of the Fd fragment (A piece), the presence of some small degree of cross-reaction cannot be excluded completely. The difficulty in precisely defining the nature of the part of the molecule that might be involved in this cross-reactivity is in part due to its poor antigenicity (immunogenicity) which was previously suggested by the close antigenic similarity among the light chains, the Fab fragment of IgG, and the IgA and IgM proteins when the appropriate antisera were used (11, 25). Consequently, it seems likely that other techniques will be required to establish precisely the nature of the non-light chain piece of the Fab fragment, and to define its possible relationship to the heavy chain.

Additional information was obtained by immunoelectrophoretic analyses. The pepsin digest reacted only weakly on immunoelectrophoresis and had a mobility similar to the native protein. Following papain digestion, as shown in Fig. 4, the protein yielded a major arc similar in mobility to the native protein and an additional, more rapidly migrating arc which gave a reaction of partial identity with the former, but was antigenically deficient. These arcs were seen with antisera to IgG, to the patient's protein, and to the Fc fragment. A similar arc (F′) has previously been described by Fahey and others in papain digests of IgG and was also seen by Osserman and by us in normal Fc fragments (23, 5 *a*, 5 *b*). In certain instances, a faint third line was visible to the cathodal side of the point of intersection of the two major arcs but its precise nature remains to be determined. It is of some interest to mention that studies by Osserman and confirmed by us have demonstrated that the heavy chain protein from one subject (Zu.) appeared to be antigenically deficient in lacking this faster migrating F′ arc (5 *a*, 5 *b*), and recent studies (35 *a*, 35 *b*, 36–38) have demonstrated that it belongs to a different subclass of IgG heavy chains.

Antisera were also prepared to the papain and pepsin digests. The former were identical with those against the native protein. Those against the pepsin digest were weak and gave a reaction of identity with the native protein, papain or pepsin digest, and IgG. When used on immunoelectrophoresis against the papain digest, this antiserum gave a single precipitin line with two peaks showing a reaction of almost complete identity, thus suggesting that the pepsin digest contains groups common to both peaks. These antisera to the pepsin digest failed to react with light chains, and also failed to react with the Fab fragment.

Since it seemed possible that the faster line might be due to a contaminant present in these proteins, many control studies were carried out with a variety of isolated β-globulins, especially transferrin, but none of these reacted in a manner similar to these lines.

Chemical characterization: Peptides obtained by trypsin digestion of the serum and urine proteins after oxidation with performic acid were separated by chromatography and high voltage electrophoresis and compared to those obtained from similarly treated G immunoglobulin, heavy chains, light chains, and Fab and Fc fragments of IgG. Fig. 5 *a* shows the appearance of the peptide map of the urine globulin and demonstrates the striking similarity to the patterns obtained with Fc fragments (Fig. 5 *b*). A total of about 25 dark spots

TABLE III

Amino Acid Composition of Urine and Serum Proteins Compared to G Immunoglobulin
All Data as gm amino acid residue per 100 gm protein.

Amino acid	Urinary protein	Serum protein	Normal pooled human γ-globulin
Lysine	9.53	9.73	7.03
Histidine	3.38	3.49	2.26
Arginine	3.88	3.96	3.99
Glucosamine*	2.88	3.20	Present
Aspartic acid	9.14	9.02	7.80
Threonine	6.23	6.22	7.54
Serine	6.03	6.04	9.71
Glutamic acid	12.84	12.64	10.96
Proline	8.58	8.62	6.67
Glycine	2.29	2.32	3.39
Alanine	1.84	1.88	3.24
Half cystine	2.34	2.20	1.92
Valine	7.92	7.59	7.98
Methionine	1.48	1.51	0.79
Isoleucine	1.88	1.88	2.23
Leucine	7.05	7.03	7.39
Tyrosine	5.12	4.68	6.08
Phenylalanine	4.08	3.83	4.28
Tryptophan			3.12

* Uncorrected for destruction during 24 hour hydrolysis in 6 N HCl.

was seen, most of which were common to both. Only one major and several faint spots marked by the circle were unique to the patient's protein when compared to the Fc fragment from a subject of the same Gm type. Comparison to the heavy chains proved more difficult because of uncertainty regarding their purity. However, here too the over-all similarity of the major spots was striking although several spots present in the heavy chain were lacking in the patient's protein (Fig. 5 *c*). Some of these could be identified in peptide maps of the Fab fragment. In contrast, light chains and Fab fragments were strikingly different as were heavy chains from IgM and IgA fractions which resembled this pattern only in a small number of spots (26, 27). The patterns of the serum and urine

proteins were virtually identical. Fingerprint analyses of these proteins and fragments by Putnam (22) are in agreement with these findings. The number of peptide spots is about half of the number expected on the basis of a molecular weight of 51,000 and the arginine and lysine content determined by Putnam (22). This suggests the presence of two similar half-molecules, an interpretation in agreement with the fall in molecular weight after reduction and alkylation.

Table III lists the amino acid analysis of the serum and urine proteins kindly performed by Dr. F. Putnam. These are quite similar to those obtained with the F fraction of a myeloma protein (22). On the basis of the minimum molecular weight calculated from the content of various amino acids, the molecule likewise appears to be a dimer.

DISCUSSION

Studies of the structural units of immunoglobulins have rapidly progressed in recent years in spite of two major limitations imposed by the products analyzed. One of these is the fact that the chains are produced by relatively drastic procedures which have been shown to alter their properties significantly in some, if not all instances. The other is the difficulty in preparing heavy chains in a state of purity when rigid immunologic criteria are used to characterize them (28). Consequently, studies of naturally occurring polypeptide chains produced in certain lymphoproliferative disorders have been quite useful. However, these too are limited by the fact that these proteins differ from the heterogeneous population of normal γ-globulin, either in representing a selective increase in a normal subfraction or, alternatively, because they are abnormal proteins which are similar to but not identical with those normally present. With these reservations in mind, and on the basis of prior studies with paraproteins, the use of pathologic "heavy chains" as models of the normally occurring counterpart appears justified. However, a number of difficulties encountered in these studies preclude a definitive answer concerning the precise relationship of this protein to the heavy chain of G immunoglobulin. The major ones are the difficulties in preparing biologically active immunochemically pure heavy chains from IgG proteins and difficulties in characterizing the part of the heavy chain presumed to reside in the Fab fragment after papain digestion. This piece, the Fd fragment previously called the A piece (29) is of utmost biologic importance since it appears to contain the antibody-combining site, but is only poorly immunogenic and difficult to isolate.

The findings reported in this study clearly demonstrate a close relationship between the patient's protein and the Fc fragment of G immunoglobulin. The genetic Gm factors are present in both. Studies with most antisera have shown a close similarity between them, and amino acid analyses were very similar. Reduction and alkylation of both yielded products with molecular weights of about 26,000, and peptide maps revealed almost identical peptide spots in both

proteins. The reason for the large amount of carbohydrate remains to be explained. One possibility is that all the carbohydrate of G immunoglobulin is normally present in one-half of the Fc fragment, having a molecular weight of about 25,000 and that this patient's protein represents a dimer of this fraction. One would then have to assume that the other half of the Fc fragment had a similar amino acid composition but lacked the carbohydrate. Alternatively, one might postulate the existence of two types of IgG's which differ only in that one has a Fc fragment rich in carbohydrate and the other is devoid of it. No evidence favoring such a concept is available either from studies of normal γ-globulins or of myeloma proteins. On the basis of previously published values for G immunoglobulins, this fragment would then be expected to contain 7 per cent hexose, 7 per cent hexosamine, 2 per cent fucose, and 1 per cent sialic acid, values not too different from those found. However, these values are significantly higher than those obtained with artificially prepared Fc fragments and may reflect the fact that this is a paraprotein.

Against the idea that this is simply the fast fragment were the findings with one antiserum of a small degree of cross-reaction with the antigen-binding (Fab) fragment through the non-light chain piece. Because of the weak antigenicity of this fragment, these studies were difficult to reproduce, and the possibility that trace contaminants were present could not be excluded with absolute certainty. The reason why the sedimentation coefficient was slightly higher than that of the Fc fragment and was similar to it only after further papain digestion which removed the F′ piece is not apparent, but may simply reflect differences in tertiary structure of a naturally formed protein compared to an artificially produced one.

While precise identity with the Fc fragment cannot be established with absolute certainty, its similarity to the product currently referred to as the heavy chain seems even less likely. The most striking discrepancy was the dissociation of the patient's protein into two equal halves by reduction and alkylation, a procedure which is used to prepare complete heavy chains of 55,000 molecular weight from G immunoglobulin and does not result in further breakdown to smaller fragments (10). The minor differences in fingerprint patterns are difficult to evaluate since the postulated part of the heavy chain in the Fab fragment appears to yield only a small number (3 to 4) of spots in the peptide maps in addition to those due to light chains (15). Studies of the other heavy chain proteins have contributed little further information (5 b); in fact, one of these (Zu.) is antigenically different and appears to belong to a different antigenic class of heavy chain (37).

While these findings suggest that this protein corresponds not to the entire normal heavy chain, but rather to a major part which contains the antigenic determinants and the carbohydrate moiety and carries Gm factors, several other possibilities must be considered. The least likely appears to be that these

differences between this protein and the heavy chain, which are greater than those usually encountered with paraproteins, are due to the fact that this is a product of malignant plasma cells. Another possibility is that the susceptibility to reductive cleavage is altered when the heavy chain is incorporated into the complete γ-globulin molecule; but little precedence appears to exist for such a concept. An alternative explanation, which bears precedence in the case of myeloma proteins and BJPs, is the possibility that there exists heterogeneity of heavy chains similar to that previously noted for the light chains of different immunoglobulin or myeloma proteins and that this protein is related to only one subfraction of the heavy chains. The possibility of this explanation is favored by the recent observations (30, 35 *a*, 35 *b*, 36–38) of differences in the heavy chains of different myeloma proteins and in the heavy chains of the five patients with heavy chain disease (5 *a*, 5 *b*). However, the differences between these proteins and heavy chains appear to be greater than could be accounted for on this basis.

From a theoretical point of view, the precise relation to currently defined structural units is of utmost importance in clearly elucidating the structure of G immunoglobulin and in resolving some of the difficulties and inconsistencies that have arisen as a consequence of the currently favored four chain model. If this protein indeed represents the crystalline (Fc) fragment, it seems likely that this is a natural structural unit of G immunoglobulin since it seems probable that a disorder of protein synthesis would give rise to a complete structural unit as it does in the case of the BJPs rather than an artificial subunit or an incomplete peptide chain. Under those circumstances, a six chain model composed of two light chains, two heavy chains corresponding to the monomeric units of the Fc fragment and unique for each immunoglobulin, and two chains equivalent to the Fd fragment (A piece) (29), would have to be postulated. It seems possible, and even attractive, to think that this part of the molecule, which is only poorly understood and yields only a small number of peptide spots after tryptic hydrolysis, may be in its gross properties common to all the immunoglobulins, but that it may vary in actual composition for each antibody and thus permit the degree of flexibility required for the great variability needed in antibody structure. Such a piece, possibly closely related to the light chains in structure, may well explain some of the discrepancies that have arisen as a result of genetic studies of rabbit, mouse, and human immunoglobulins (31–33). An additional observation suggesting the existence of an unusual bond between the Fc fragment and the Fd fragment is its unique susceptibility to a variety of proteolytic enzymes which have been used to degrade γ-globulin (34). All of these appear to cleave the molecule at this site. This finding suggests some unique structure for this part of the γ-globulin molecule and points to the existence of two distinct subunits which together are currently called the heavy chain.

Regardless of the ultimate answers to these questions, studies of this protein have clearly demonstrated its close relation to the part of the molecule that is responsible for most of the characteristics currently ascribed to the heavy chain and suggest the eventual discovery of similar proteins related to IgA and IgM fractions.

SUMMARY

1. Detailed physical, chemical, and immunologic studies of a protein closely related to the Fc fragment and heavy chain of G immunoglobulin (IgG), and elaborated by a subject with a lymphoproliferative disorder are presented.

2. The protein, which has a molecular weight of 51,000, was cleaved into two half molecules by reduction and alkylation.

3. The protein has few if any of the antigenic determinants of the antigen-binding (Fab) papain fragment of IgG, and has a striking similarity in its antigenic properties to the Fc fragment.

4. Fingerprint patterns resemble those of the crystallizable (Fc) fragment, and lack several peptides found in the heavy chain.

5. These findings suggest that the Fc fragment may be a real structural unit of IgG, and raise the possibility of the existence of three different types of polypeptide chains in G immunoglobulin.

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