

Products of the Peptic Digestion of Human γ G-Immunoglobulin*

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ABSTRACT: Pepsin digestion of human γ G-immunoglobulin (IgG) results in four components, whose relative yield relates to conditions of incubation as well as to the nature of the substrate. Prolonged exposure to pepsin also results in changes in the chemical nature of the largest component. This change is characterized by a fall in sedimentation coefficient and progressive inability to separate constituent Fd' fragments and light chains (L chains) by Sephadex G-100 urea gel filtration. The other major degradation product of peptic digestion

bears Fc antigenic determinants only, but is distinct from the papain-obtained analog by its lower molecular weight (25,000) and lack of carbohydrate. Other components arising from peptic digestion of IgG were carbohydrate-containing material with a molecular weight of 7000 and smaller peptides presumably arising from the other portions of the Fc fragment region. The degradation of human γ G-immunoglobulin by pepsin is similar to the degradation of rabbit γ G-immunoglobulin.

Some understanding of the structure of antibodies may be obtained on degrading IgG¹ with proteolytic enzymes and examining the fragments which retain antibody activity. Fab fragments (Porter, 1959) obtained by digestion with papain bind one molecule of antigen, but two molecules of antigen are bound by F(ab')₂ fragments (Nisonoff *et al.*, 1961) which arise on peptic digestion. The products of papain (Porter, 1958) and pepsin (Nisonoff *et al.*, 1960; Utsumi and Karush, 1965) digestion have been studied mainly on rabbit IgG. Although analogous studies on human IgG have been carried out with papain (Hsiao and Putnam, 1961; Franklin, 1960; Edelman *et al.*, 1960), only scattered data are available for similar studies with pepsin. An attempt was therefore made to provide this information.

This report also illustrates some of the difficulties encountered in the isolation of fragments of constant physicochemical character when human IgG, a heterogeneous protein substrate, is subjected to the action of the relatively nonspecific enzyme pepsin. To facilitate the reader's understanding of the nomenclature used in this report models of the IgG molecule with the

pertinent enzymatically produced fragments are shown on Figure 1.

Materials and Methods

Human Immunoglobulin G. Experiments were performed on preparation L 2351 (Mann), chromatographically isolated from pooled human plasma and showing only one precipitin band on immunoelectrophoresis against a number of potent antisera to human serum. Other preparations used were pooled human fraction II (Hyland) and IgG preparations of a myeloma serum (Ro) and a normal serum (Le), prepared by 18% Na₂SO₄ precipitation followed by DEAE-cellulose chromatography.

Antisera. Antisera to human serum, IgG, Fab fragment, and Fc fragment were purchased from Hyland Laboratories. Antisera to isolated L chains and H chains were prepared in this laboratory. The latter antisera were absorbed with appropriate fractions to yield a precipitin band only against the requisite antigen.

Pepsin Digestion. Human IgG was dissolved in 0.2 M acetate buffer, pH 4.5, and incubated with pepsin (three times crystalline, Nutritional Biochemicals, Co.), 2% with respect to substrate concentration, for various intervals at 37°. The reaction was stopped by adjusting to pH 8 with solid Tris. The digests were centrifuged to remove suspended material. With IgG (Mann) 1 g was used in each experiment. With other IgG preparations 500 mg were routinely incubated.

Papain Digestion. This was carried out according to Porter (1958). IgG (1 g, Mann) was incubated with 1% papain (Worthington Biochemical Corp.).

Chemical Procedures. The material was reduced and alkylated as described by Edelman *et al.* (1960). Sulfitolysis with 0.25 M Na₂SO₃ in the presence of 0.005 M Cu(NO₃)₂ was performed as described by Franek and Nezlin (1963). Hexose was determined by the orcinol

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¹ We have followed the recommendations of the World Health Organization Committee on Nomenclature (*Bull. World Health Organ.* 30, 447 (1964)) by using the following terms and abbreviations: IgG for γ -G-immunoglobulin, also known as 7S γ -globulin; IgM for γ -M-immunoglobulin, also known as 19S γ -globulin; IgA for γ -A-immunoglobulin, also known as γ 1A-globulin; H chain for heavy chain; L chain for light chain. The nomenclature used for fragments obtained by proteolytic action is explained in Figure 1.

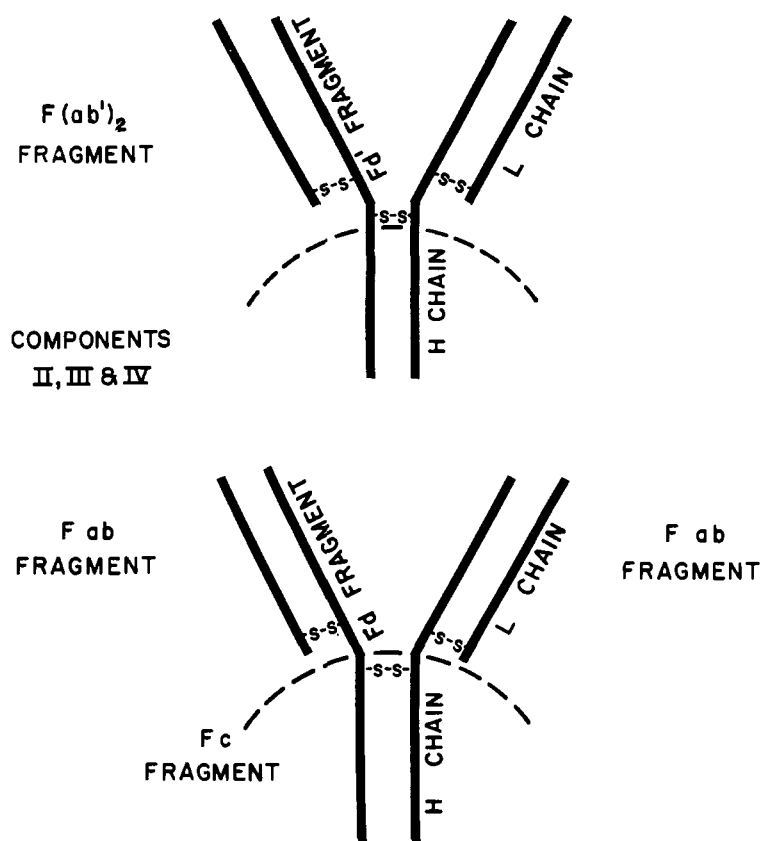


FIGURE 1: Model of IgG molecule. (Top) Degradation by pepsin leads to F(ab')₂ fragment, comprising two L chains and two Fd' fragments linked by at least three disulfide bonds, and to three other components located on the carboxyl-terminal end of the H chain. (Bottom) Degradation by papain in presence of low concentrations of cysteine leads to two Fab fragments, containing L chain and Fd fragment, and the Fc fragment.

method (Winzler, 1955).

CM-cellulose (Selectacel, Carl Schleidher and Schuell Co., lot 1342, 0.78 mequiv/g-capacity) chromatography was carried in columns of 25×2.5 cm connected to a three-chambered mixing device for gradient elution. Sephadex gel (particle size 40–120 μ , Pharmacia Co.) filtration was done by downward vertical elution. Urea for buffer solutions was purified by mixed-bed resin ion-exchange chromatography.

Starch Gel Electrophoresis. This was carried out in vertical trays in 8 M urea and 0.035 M glycine, pH 8.8, for 18 hr at 5 v/cm as described by Cohen and Porter (1964).

Sedimentation Coefficients and Molecular Weight Determinations. Determinations were made in the Spinco Model E analytical ultracentrifuge at 20°. Sedimentation coefficients were determined from runs made with at least three different concentrations of sample. Values were corrected for standard conditions in the usual manner. Molecular weights were determined on approximately 0.5% samples with schlieren optics by the approach to equilibrium method. Photographic plates were measured on a Nikon comparator and dc/dx and c_m values obtained from runs at different speeds were plotted according to the method of Traut-

man (1956) as modified by Erlander and Foster (1959). Where indicated molecular weight determinations by sedimentation equilibrium were also performed on approximately 0.1% samples in short columns with interference optics (Van Holde and Baldwin, 1958).

Results

Separation of Fragments Obtained on Digestion with Pepsin. Peptic digests of samples of human IgG, incubated for 4 hr at 37° at pH 4.5, were filtered through a Sephadex G-75 column (120 \times 3 cm). Figure 2 shows an elution pattern comprised of four major components, obtained with a peptic digest of 500 mg of IgG (Mann). This general pattern was also observed with peptic digests of the other three IgG preparations tested. Component I was found to be the F(ab')₂ fragment and will be referred to by this name wherever appropriate. It was usually contaminated with small amounts of apparently undigested IgG. Component II was antigenically related to the Fc fragment, but was of considerably lower molecular weight than the fragment obtained with papain. Component III was the major carbohydrate-containing moiety and com-

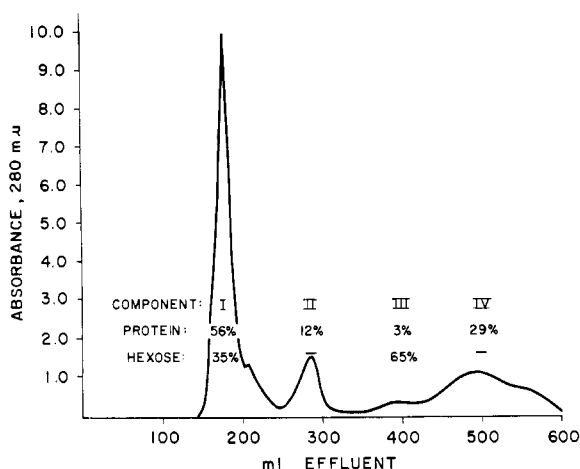


FIGURE 2: Sephadex G-75 gel filtration of 500 mg of IgG (Mann), digested by 10 mg of pepsin at pH 4.5 for 4 hr at 37°. The relative concentration of the four separate components calculated by determination of areas swept out by absorbance measurements. Hexose, determined on summation of values for each fraction.

ponent IV was comprised of low molecular weight polypeptides.

The digest of IgG (Mann) contained approximately 15% insoluble material, whereas digests prepared from other preparations were relatively clear at the end of the 4-hr incubation period. As can be seen from Table I,

TABLE I: Relative Yield of Components Derived from Pepsin Digestion of Human IgG.

| IgG Preparations ^a | Components | | | |
|-------------------------------|------------|----|-----|----|
| | I | II | III | IV |
| IgG (Mann) | 56 | 12 | 3 | 29 |
| Human fraction II (Hyland) | 71 | 10 | 5 | 15 |
| IgG (myeloma Ro) | 66 | 10 | 2 | 22 |
| IgG (normal Le) | 56 | 12 | 6 | 27 |
| Theory ^b | 73 | 17 | 5 | 6 |

^a Preparations were incubated for 4 hr at 37° in pH 4.5 acetate buffer with pepsin (2% relative to protein concentration). Eluates from Sephadex G-75 gel filtration were measured for absorbance at 278 mμ, plotted, and areas under the peaks were taken as the amount of material. Biuret reactions and absorbance at 278 mμ did not deviate by more than 10%. ^b Theoretical yield calculations were based on average molecular weights of 145,000 for IgG, 106,000 for component I, 25,000 for component II, and 7000 for component III. Component IV content was estimated by summing up components I, II, and III and subtracting from 145,000.

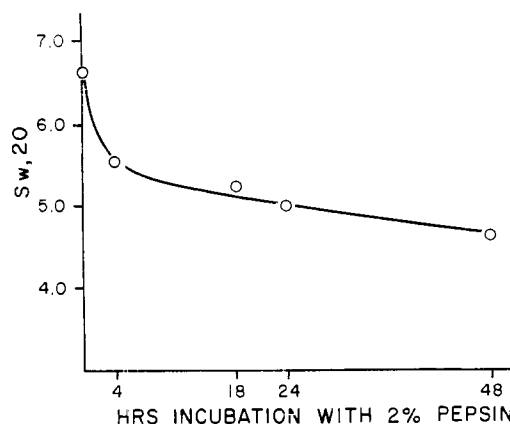


FIGURE 3: The dependence of the sedimentation coefficient of F(ab')₂ fragments on the time allowed for digestion of IgG (Mann) by pepsin.

the relative yield of the four components of the pepsin digest was subject to considerable variation. Particularly striking were the differences among the low molecular weight polypeptides comprising component IV. Although fraction II (Hyland) yielded the least amount of component IV, this amount was still twice that expected from theory. As exposure of IgG to 6 M urea prior to pepsin digestion also leads to a large increase in the yield of component IV, it would appear that the greater the conformational change of IgG, the greater the yield of component IV and consequently the smaller the yield of components I and II.

Effect of Duration of Incubation with Pepsin on Recovery of the Four Components. These experiments were carried out with IgG (Mann) only, using a Sephadex G-75 gel column (120 × 6 cm) with equal amounts of 0.15 M Tris buffer and physiologic saline (pH 8) as the eluent. Table II shows that prolonged exposure to pepsin resulted in decreased yields of F(ab')₂ fragments and of component II, with a consequent in-

TABLE II: Relative Yield of Components Derived from Pepsin Digestion of Human IgG^a as a Function of Time of Incubation.

| Incubn (hr) | Components | | | |
|-------------|------------|----|-----|----|
| | I | II | III | IV |
| 4 | 56 | 12 | 3 | 29 |
| 24 | 56 | 9 | 3 | 31 |
| 48 | 45 | 3 | 3 | 49 |

^a IgG (Mann) was incubated for the time intervals indicated at 37° in pH 4.5 acetate buffer with pepsin (2% relative to protein concentration). Eluates from Sephadex G-75 gel filtration were measured for absorbance at 278 mμ, plotted, and areas under the peaks were taken as the amount of material.

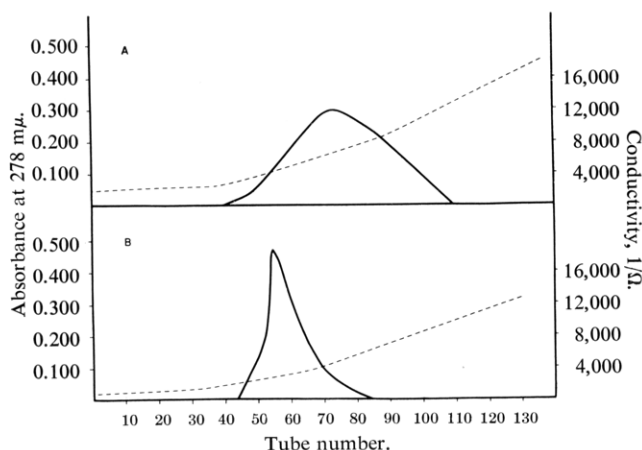


FIGURE 4: CM-cellulose chromatography of $F(ab')_2$ fragments derived from IgG (Mann). (A) Elution performed in the absence of urea. (B) Elution performed in the presence of 6 M urea. $F(ab')_2$ fragments prepared by incubation with pepsin for 4 hr at 37° at pH 4.5 and purified by Sephadex G-100 (component I). Gradients prepared in a three-chamber Varigard (Buchler Co.) from 0.02, 0.1, and 1.0 M sodium acetate at pH 5.5. Solid lines: absorbance at 278 m μ ; dashed lines: conductivity.

crease of low molecular weight polypeptides. Component II appeared to be virtually destroyed after a 48-hr exposure to pepsin. While the recovery of $F(ab')_2$ fragments was only moderately affected by prolonged exposure of IgG to pepsin, the following section details other changes in the character of this fragment.

Examination of $F(ab')_2$ Fragments in the Analytical Ultracentrifuge. $F(ab')_2$ fragments, prepared from IgG (Mann), when examined in the analytical ultracentrifuge exhibited a sedimentation coefficient dependent on the length of time of enzymatic incubation (Figure 3). The value of 5.5 S for the 4-hr incubation period was probably the result of incomplete degradation of intact IgG. Admixture with such products was actually demonstrable by agar gel diffusion studies, as component I derived from short-term incubation experiments invariably reacted with Fc antisera. Refiltration of this material through Sephadex G-100 permitted separation of undigested or partially digested IgG from $F(ab')_2$ fragments. The decline in sedimentation coefficients with respect to incubation time may have been caused by preferential digestion of peripheral peptides. As will be shown in a later section, these peptides seemed to have originated from the H-chain portion of $F(ab')_2$ fragments. As constant sedimentation coefficients were not reached even after 48 hr of incubation, molecular weight determinations of $F(ab')_2$ fragments were not performed.

Subfractionation of $F(ab')_2$ Fragments. In view of the evidence that Fab fragments obtained by papain digestion may be resolved into two chromatographically distinct fractions (Porter, 1958), experiments were

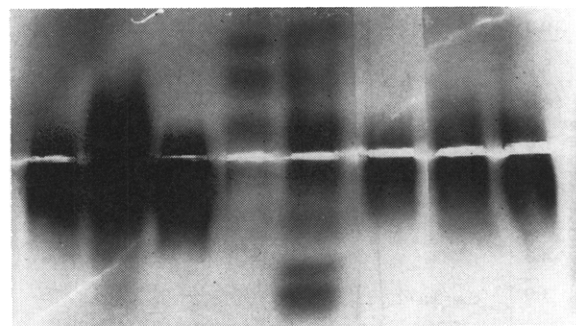


FIGURE 5: Starch gel electrophoresis in urea glycine, pH 8.8 (left to right): $F(ab')_2$ fragment preparations of IgG (Mann), fraction II (Hyland), and IgG (Mann); component II preparations of IgG (Mann) and fraction II (Hyland); three different Fd' preparations of IgG (Mann). Anode at bottom.

undertaken to probe for a similar heterogeneity in $F(ab')_2$ fragments. $F(ab')_2$ fragments obtained by a 4-hr pepsin digestion were further fractionated by CM-cellulose chromatography. As shown in Figure 4, $F(ab')_2$ fragments failed to resolve into two chromatographically distinct fractions. In the absence of urea they formed a broad peak between 3000 and 12,000 1/ Ω . A sharper but skewed peak between 2000 and 6000 1/ Ω was obtained in the presence of 6 M urea. Human Fab fragments and intact human IgG were also fractionated on the same column in the presence of 6 M urea. As anticipated, Fab fragments eluted under two distinct peaks with maxima at 900 and 3000 1/ Ω . Approximately 90% of human IgG eluted under a single peak between 2000 and 3500 1/ Ω , but the remaining 10% trailed over a considerable number of tubes up to 7000 1/ Ω .

Starch gel electrophoresis patterns of two $F(ab')_2$ fragment preparations of IgG (Mann) and one of fraction II (Hyland) are shown in Figure 5. The Hyland preparation appeared to be more heterogeneous than the Mann preparation.

Conversion of $F(ab')_2$ Fragments to Constituent Fd' Fragments and L Chains. Sulfitolysis of the $F(ab')_2$ fragment followed by Sephadex G-100 gel filtration in the presence of 6 M urea and 0.1 N acetic acid resulted in the recovery of two fractions, presumably constituting Fd' fragments under the first peak and L chains under the second. Based on a molecular weight of 31,000 for Fd' fragments (Heimer, 1966) and 22,000 for L chains, a ratio of 3 parts peak I:2 parts peak II was to be expected. As can be seen in Table III the expected distribution was found only with material obtained from fraction II (Hyland) and IgG (normal, Le). Yields deviating by 50% were found with preparations of IgG (Mann) and deviating by 75% with preparations of IgG (myeloma, Ro). There also was a decided trend toward inversion of the 3:2 ratio with increased duration of the pepsin incubation.

An extensive investigation into the anomalous distribution of Fd' fragment and L chain, particularly

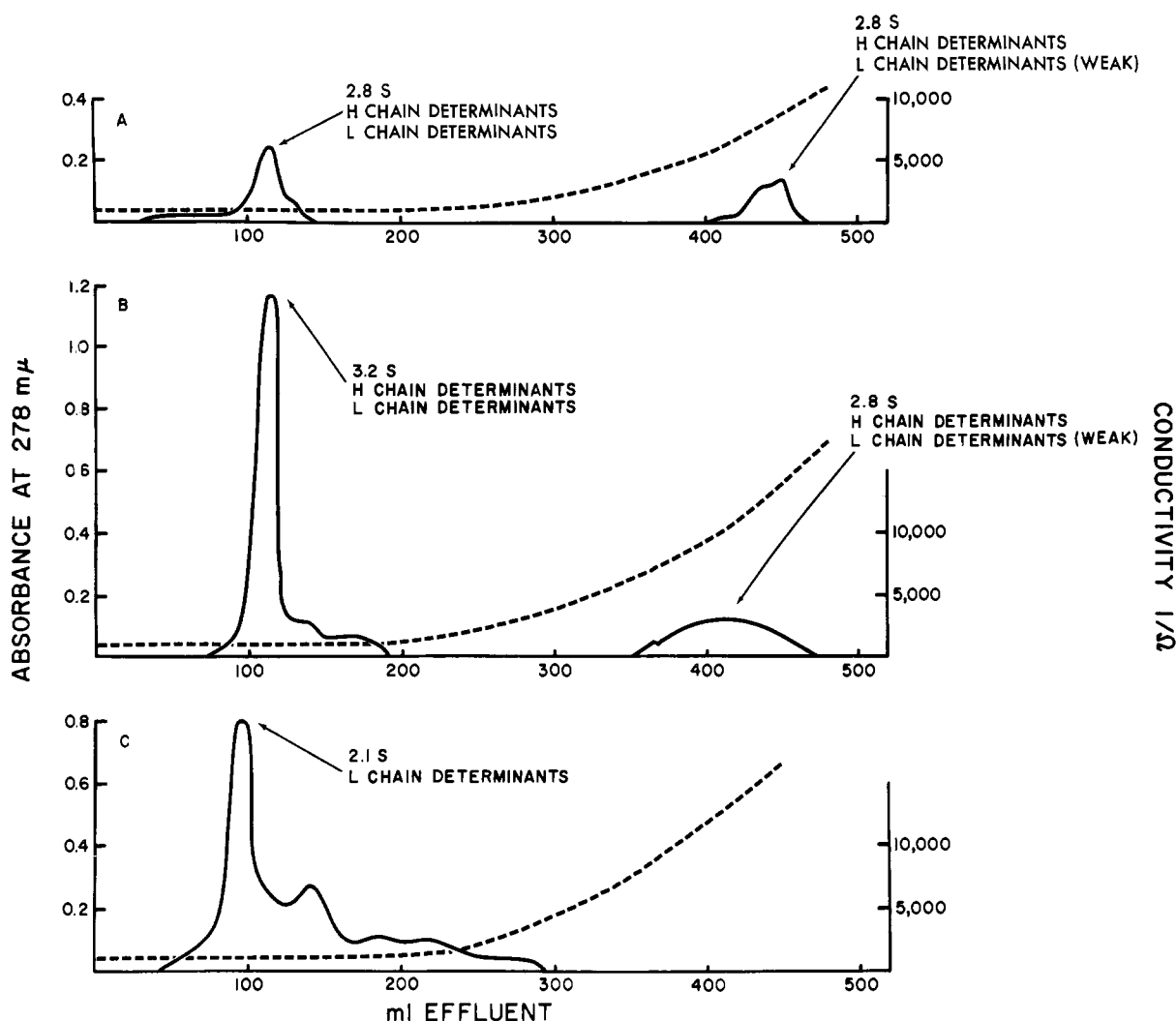


FIGURE 6: A comparison of CM-cellulose chromatograms of peaks I (A) and II (B) obtained from Sephadex G-100 gel filtration of sulfitolyzed $F(ab')_2$ fragment and of reference L chain (C). Chromatography performed on 3×40 cm columns in presence of 6 M urea with gradient elution at pH 6.0. Solid lines: absorbance at 278 mμ; dashed lines: conductivity.

with IgG (Mann), revealed that peak II of the Sephadex G-100 6 M urea filtration was actually greatly contaminated with Fd' fragments. This contamination became evident on studying peaks I and II by CM-cellulose chromatography and comparing the chromatograms with those of reference L chains, prepared from IgG (Mann) by sulfitolysis in the absence of pepsin. As seen in Figure 6, chromatography of peaks I and II yielded two components each, which although different in relative yield, emerged in similar positions. Reference L chain, however, lacked the component eluting at higher ionic strength. Antigenic analysis with anti-L-chain sera revealed a strong reaction of the material eluting at low ionic strength, but a weak reaction of the material eluting at high ionic strength. H-chain determinants were detected in all chromatographic fractions, except those prepared from reference L chain. Starch gel electrophoresis failed to show the

L-chain contamination of peak I (Figure 5). The sedimentation coefficient, established in 0.15 M phosphate buffer, pH 7.0, of both chromatographic components obtained from peak I was 2.8 S. Peak II, however, yielded one material of 3.2 S and one of 2.8 S. Reference L chain was 2.1 S. A likely explanation for the high sedimentation coefficients of peak II is that the 3.2 S material represents dimers, composed most likely of L chain and Fd' fragment. A Fd' -fragment, L-chain hybrid has been described for products obtained from rabbit IgG (Roholt *et al.*, 1966). This suggestion is supported by sedimentation velocity analysis in 1 M acetic acid, which gave 2.4 S, *i.e.*, halfway between 2.1 S for L chain and 2.8 S for Fd' fragment.

Characterization of Component II. Emerging on Sephadex G-75 gel filtration (see Figure 2) behind the $F(ab')_2$ fragment, component II appeared to be free

TABLE III: Relative Yield of Peaks I and II of Sephadex G-100 Gel Filtration of $F(ab')_2$ Fragment Preparations Subjected to Sulfitolysis.

| Source of $F(ab')_2$ Fragment ^a | Incubn | | Peak II |
|---|--------------|-----------|------------|
| | Time (hr) | Peak I | |
| Fraction II (Hyland) | 4 | 58 | 42 |
| IgG (myeloma, Ro) | 4 | 16 | 84 |
| IgG (normal, Le) | 4 | 56 | 44 |
| IgG (Mann) | 4 | 30 | 70 |
| IgG (Mann) | 24 | 25 | 75 |
| IgG (Mann) | 48 | 19 | 81 |

^a Purified $F(ab')_2$ fragments were sulfitolyzed and filtered through a 120×1.5 cm column of Sephadex G-100 with 0.1 N acetic acid in 6 M urea. Eluates were measured for absorbance at 278 m μ , plotted, and areas under the two peaks were taken as the amount of material.

of Fab fragment contamination when analyzed by immunodiffusion. It reacted only with antibody to Fc fragment. Component II exhibited many distinct bands on starch gel electrophoresis, and patterns of preparations obtained from IgG (Mann) and fraction II (Hyland) are shown in Figure 5.

Sedimentation velocity analysis in the ultracentrifuge gave 2.4 S in neutral buffers, but 1.7 S in 1 N acetic acid. These two values were unaffected by mercaptoethanol. Molecular weight determinations by the approach to equilibrium using short columns gave a molecular weight of 12,000 in 1 N acetic acid. A Trautman plot obtained from runs with a concentration of 5 mg/ml in 0.1 M phosphate buffer (pH 7) was linear throughout and gave a weight-average molecular weight of 25,000. The partial molar volume in these calculations was assumed to be 0.73 (Small and Lamm, 1966). It thus appears that component II is a dimer of non-covalently linked polypeptide chains whose composition, at least judging from starch gel electrophoresis patterns, appears to be quite heterogeneous.

Fragments of Low Molecular Weight. Component III contained approximately 65% of the total hexose of IgG. When concentrated and repurified by Sephadex G-75 filtration followed by filtration through Bio-Gel P-2, a material was obtained which sedimented as a single peak at 0.9 S in the ultracentrifuge. Based on diffusion measurements made on the same run ($D = 10 \times 10^{-7}$ cm²/sec), and an assumed $\bar{v} = 0.70$, the estimated molecular weight was 7000. This material failed to react with any of the antisera listed in the Methods section.

The polypeptides recovered in component IV took in a broad spectrum of molecular weights, from

probably 5000 on downward. The number and molecular weight distribution of these carbohydrate-free peptides was not determined. Component IV preparations failed to react with all antisera to human IgG in immunodiffusion tests.

Discussion

While giving rise to distinct fragments which may vary somewhat from preparation to preparation, a number of parameters condition the outcome of the peptic digestion of human IgG. As IgG is heterogeneous with respect to chemical composition and is also comprised of molecules in different conformational states, it is subject to enzymatic attack at positions which might vary from molecule to molecule. This situation is further complicated by the relative nonspecificity of pepsin and the rather arbitrary conditions chosen for the enzymatic reaction. It is therefore surprising, as already pointed out by Hsiao and Putnam (1961) in the context of the papain digestion of IgG, that the cleavage reaction always leads to rather well-defined fragments. In the results reported herein, fragments were obtained sedimenting at approximately 5, 2.4, and 0.9 S. There also were low molecular weight breakdown products. Differences in the yields of these fragments, however, did occur and they appeared to relate mainly to the degree of retention of the native conformational state of the preparation. As expected, the number and the severity of the manipulative steps used in the isolation of the substrate appear to dictate susceptibility to pepsin digestion.

The $F(ab')_2$ fragments obtained from human IgG vary in their sedimentation characteristics in a manner which suggests that prolonged exposure to pepsin results in further cleavage, probably at the H-chain portion of the $F(ab')_2$ fragments. This may account for the observed decrease of the sedimentation coefficient and for the concomitant inability to separate the Fd' fragment from the L chain by Sephadex gel filtration. Separation by this method is feasible only when the two components differ sufficiently from one another with respect to molecular weight. Our study also shows that controlling the duration of the pepsin incubation at 4 hr does not guarantee uniform $F(ab')_2$ fragments. Cleavage at the H-chain site must also be dictated by the nature of the starting material, for as shown in Table III, IgG (normal, Le) yielded the theoretically expected amount of L chain and Fd' fragment, but similarly exposed IgG (myeloma, Ro) was at the opposite end of the distribution, with Fd' fragment and L chain being nearly inseparable in Sephadex G-100. Additionally, our experiments suggest that even under optimal conditions neither Sephadex gel filtration, nor CM-cellulose and not even a combination of the two techniques lead to a complete separation of Fd' fragment and L chain.

Separation of L chain from Fd fragment on Sephadex G-100 was always less successful when papain was substituted for pepsin, presumably because papain splits the H chain closer to its N terminus, yielding

Fd fragments of smaller size. Fleischman *et al.* (1963) have found the Fd fragment of rabbit IgG to have a molecular weight of 22,000 and it is likely that its pepsin-obtained counterpart in the rabbit also exceeds 22,000 (Jaquet and Cebra, 1965). Our inability to isolate sufficient amounts of Fd fragment has frustrated a comparison between Fd' fragment and Fd fragment of human IgG. The two may differ with respect to a number of peptides (Fougereau and Edelman, 1965).

Interesting differences exist between the largest fragments obtained from IgG by pepsin and papain digestion. Unlike Fab fragments, F(ab')₂ fragments cannot be resolved into two separate components by CM-cellulose chromatography. As the Fab dimer of rabbit IgG, obtained by digestion with water-insoluble papain in the absence of a mercaptan, can also be resolved into two chromatographically distinct components (Jaquet and Cebra, 1965), it would appear that chromatographic heterogeneity is suppressed by presence of the peptides which lie between the carboxyl-terminal end of the Fd and Fd' fragments. This assumes, however, that neither papain nor pepsin alter N-terminal sequences.

F(ab')₂ fragments of human IgG contained approximately one-third of the carbohydrate of the native molecule (Figure 2). This agrees with the findings of Utsumi and Karush (1965) on rabbit F(ab')₂ fragments. As rabbit Fab fragments also have significant amounts of carbohydrate (Goodman, 1965), it would appear that as in the rabbit there must be at least two separate positions bearing carbohydrate moieties on the human H chain. L chains lack significant amounts of carbohydrate.

Component II, being antigenically related to the Fc fragment but about one-half in molecular weight, may resemble the previously described F'c (Poulik, 1966) or F' fragment (Fahey and Askonas, 1962) in a number of ways, but appears to differ in that its sedimentation coefficient of 2.4 S is somewhat higher than the 2.1 S reported by Poulik (1966). It appears that component II is analogous to the Pep-III' fragment obtained on peptic fragmentation of rabbit IgG (Utsumi and Karush, 1965). The latter preparation was described to be a dimer with a molecular weight of 27,000, lacking carbohydrate and interchain disulfide bonds. Moreover, the emergence of component II on Sephadex G-75 gel filtration and the lability to pepsin digestion also appears to be strikingly similar to Pep-III'. Beyond testing for Fc and Fab antigenic determinants, a direct antigenic comparison between isolated Fc fragments and component II has not been made, but the lack of carbohydrate moieties and the considerably lower molecular weight clearly indicate that, as with rabbit IgG (Goodman and Gross, 1963), component II must be at least part of the Fc fragment. Its position within the heavy chain could not be established with any certainty. Its relationship to a human urinary material named Fc' fragment (Turner and Rowe, 1966) was not probed.

Component IV is probably a mixture of breakdown products recruited from every portion of the IgG

molecule. This was deduced from its large and variable yield. We are unable to assign a region on the H chain to peptides which invariably emerge in this component. If such specific peptides exist, they may derive from a number of positions along the H chain. Based on molecular weight determinations of H chain, Fd' fragment, component II, and the carbohydrate-containing peptides, component IV might comprise unique peptides accounting for approximately 10% of the H chain.

It would appear from the results obtained and some of the conclusions drawn that the peptic digestion of the IgG appears to run a parallel course whether the source of the immunoglobulin be man or rabbit. The major fragments obtained on pepsin digestion of rabbit and human IgG appear to be similar in most respects. The results provide a further basis for the assumption that the presently accepted structural model of IgG is valid for both rabbit and man.

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Variability in the Specific Interactions of H and L Chains of γ G-Globulins*

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ABSTRACT: The specificity of noncovalent interactions between human γ G-globulin H- and L-polypeptide chains was investigated. A previous study demonstrated that specificity exists in these interactions in that the heavy (H) and light (L) chains of a human G-myeloma protein tend to reassociate even if other free L chains are present [Grey, H. M., and Mannik, M. (1965), *J. Exptl. Med.* 122, 619]. The current study extends these observations and shows that the preferential recombination of autologous H and L chains, if present, occurs even at 80-fold molar excess of heterolo-

gous myeloma protein L chains. On the other hand, normal γ G-globulin L chains contain populations of molecules that can replace randomly the autologous L chains of myeloma proteins from recombining with their H chains.

The percentage of such molecules varied from 23 to 73%, depending on the myeloma protein used. Natural γ G-globulin molecules that contain one κ and one λ chain are not encountered. *In vitro*, such molecules form readily between a given H chain and a pair of κ and λ chains.

The noncovalent interactions between the heavy (H) and light (L) chains¹ of γ G-globulins are sufficient to maintain the integrity of these four-chain molecules in neutral weak salt solutions so that they continue to function as specific antibody molecules and display many of the physiochemical characteristics of the native molecules (Edelman *et al.*, 1963; Olins and Edelman, 1964; Roholt *et al.*, 1964; Metzger and Mannik, 1964; Fougereau and Edelman, 1964). Specificity exists in these noncovalent bonds in that the H chains of a homogeneous γ G-globulin, either from a myeloma protein or from specific antibody, tend to recombine with their own L chains even when L chains from other homogeneous γ G-globulins are present (Grey and Mannik, 1965; Roholt *et al.*, 1965). The previous study, utilizing human G-myeloma proteins as models of homogeneous populations of molecules, showed considerable variation among L chains of various myeloma proteins in their capacity to replace autologous L chains from their interaction with H chains

(Grey and Mannik, 1965). These and other observations stimulated more detailed studies of the competitive reactions with particular emphasis on the heterogeneous population of normal γ G-globulin L chains which should contain molecules that are capable of replacing at random the autologous L chains. The data presented below show that the specificity of noncovalent interactions between autologous H and L chains of G-myeloma proteins, if present, persists in large molar excess of heterologous L chains. Furthermore, normal γ G L chains contain molecules capable of replacing at random the autologous L chains from recombining with their H chains. Deductions from the data suggest that the variable portion of L chains participates in the noncovalent interactions with H chains.

Materials and Methods

Isolation of γ G-Globulins. Myeloma proteins were isolated and immunochemically characterized as previously described (Grey and Mannik, 1965). Normal pooled γ G-globulin was isolated from fraction II (Lederle Laboratories, lot no. C-803) by DEAE-cellulose chromatography. The protein concentrations in all experiments were determined by the method of Lowry *et al.* (1951).

Preparation of H and L Chains. The H and L chains from the isolated proteins were prepared by the methods

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¹ The nomenclature for immunoglobulins and their polypeptide chains is that recommended by a conference on immunoglobulins sponsored by the World Health Organization, *Bull. World Health Organ.* 30, 447 (1964).