Action of Papain on Human Serum Globulins*

H. F. Deutsch, E. R. Stiehm, and Jane I. Morton

From the Department of Physiological Chemistry, University of Wisconsin, Madison 6, Wisconsin

(Received for publication, January 23, 1961)

Studies on the degradation of animal and human serum antibody type proteins by various proteolytic enzymes have indicated that molecules of approximately one-half and one-quarter the size of the parent 100,000-molecular weight protein may be readily formed (1-5). Porter (6) has recently shown that rabbit γ-globulin is degraded by papain into fragments of the above size range and has separated such digests into three discrete fractions by chromatography on carboxymethyl-cellulose columns.

With papain under conditions in which normal γ-globulins (7) are only partially degraded into lower molecular weight fragments, it has been found that all myeloma serum globulins examined are uniformly converted to molecules sedimenting near 3.5 S. This is in the range of the most commonly reported values for Bence-Jones proteins (8). Under analogous conditions, macroglobulins of the Waldenstrom type (9) are only partially degraded into lower molecular weight fragments, it has been found that all myeloma serum globulins examined are uniformly converted to molecules sedimenting near 3.5 S. This is in the range of the most commonly reported values for Bence-Jones proteins (8). Under analogous conditions, macroglobulins of the Waldenstrom type (9) are only partially degraded into lower molecular weight fragments.

The present studies are concerned with the properties of the products formed in the splitting of several human globulins by papain, with special reference to the myeloma proteins.

Experimental Procedure

The myeloma and macroglobulinemia proteins and normal γ-globulins utilized in this work have been described in previous reports (7, 10, 11). Solutions (1%) of these proteins in pH 5.0, 0.01 to 0.05 M sodium citrate buffer at 37° were treated with 0.1 to 1.0% of their weight of 2X crystallized mercaptapapain (Nutritional Biochemicals). No reducing agent was added to "activate" the papain. In some cases ethylenediaminetetraacetate was used in the incubation mixture at a final concentration of 1 X 10^{-2} M. The chelating agent was not an obligatory constituent of the digest but in its absence approximately 5 times the usual amount of papain was required. The digestion was allowed to proceed for varying periods at 37°, usually overnight (14 to 18 hours). Residual macroglobulins in the digests of such proteins precipitated upon dialysis against distilled water and were removed by centrifugation. The papain digestion products of multiple myeloma proteins and normal γ-pseudoglobulins usually fail to form insoluble protein on dialysis.

Electrophoretic and ultracentrifugal analyses were performed with the Spinco model H and E apparatuses, respectively. The electrophoretic analyses were performed with a 0.1 ionic strength, pH 8.6 diethylbarbiturate buffer and were carried out for approximately 180 minutes at a potential gradient near 4.5 volts cm^{-1}. The sedimentation experiments were performed at 59,780 r.p.m. in 0.2 ionic strength, pH 7.4 potassium phosphate buffer. Chromatographic experiments on 100- to 300-mg samples of protein utilized carboxymethyl- and DEAE-cellulose (12) columns of 2 X 30 cm. A constant volume gradient elution technique was utilized with pH 5.5 sodium or ammonium acetate buffer being used over a 0.01 to 0.5 M range.

The protein fractions in the chromatographic effluents were recovered by dialysis and lyophilization. Samples were taken for determination of moisture, ash, and amino acid composition. A low molecular weight papain digestion fraction was examined on a column of cross-linked dextran gel as described by Purich and Flodin (13). (Sephadex G-25 was obtained from Pharmacia Laboratories, Inc., 501 Fifth Avenue, New York 17, N. Y.) The peptides in this low molecular weight fraction were also chromatographed on a 0.9- X 40-cm column of Dowex 50-X2 (14) with a gradient from 0.2 M, pH 3.25, to 1.0 M, pH 5.1, sodium citrate buffer.

Amino acid analyses were performed by ion exchange chromatography (15) with automatic recording equipment (16). Analyses for cystine as cysteic acid were made by the procedure of Schram et al. (17) on the performic acid-oxidized proteins or peptides, with the use of the amino acid analyzer for the determination of the cystic acid.

The COOH-terminal amino acid determinations were made with crystalline carboxypeptidase which had been treated with diisopropylfluorophosphate (Worthington). Approximately 1 mg of this enzyme was incubated with 100 mg of protein for 3 to 8 hours in 0.2 M, pH 8, ammonium carbonate buffer. The experiment was conducted in the dialyzing apparatus of Craig, King, and Stracher (18), and the dialysate was concentrated in a rotary evaporator to remove water and salt. The amino acids in the residue were determined with the automatic equipment.

Sulfhydryl determinations were carried out by the spectrophotometric method of Boyer (19). Determinations of cysteine were carried out by reaction with iodoacetic acid at pH 9 in 8 M urea or 0.2 M sodium lauryl sulfate, and with iodoacetamide in 6 M guanidinium chloride, followed by chromatographic measurement of carboxymethylcysteine, under the conditions described by Cole et al. (20) for studies on hemoglobin.

Results

γ-Globulin—Digestion of this protein with 1% of its weight of papain leads to the formation of a series of degradation products. An ultracentrifuge diagram, which is shown as part of Fig. 1, reveals that the major portions of these proteins still sedimented at their original rate, i.e. 6.5 S. Prolonged digestion does not change the ratio of the components formed. In order to inhibit any further action of papain after digestion had been
carried out for the desired length of time, iodoacetamide was added to the digestion mixture to a concentration of 0.02 M and the pH was adjusted to 7.5 to 8.0. After a 1- to 2-hour incubation at 37°, the mixture was dialyzed against several changes of distilled water for 12 to 24 hours and the solution was then lyophilized. Upon treatment of a 1% solution of the digested material (without removal of papain) with 0.01 M 2-mercaptoethanol in 0.2 ionic strength, pH 7.4 potassium phosphate buffer, essentially all of the protein was converted to molecules sedimenting near 3.5 S (see Fig. 1). A similar result has been obtained with pepsin-digested rabbit \( \gamma \)-globulin by Nisonoff et al. (21). Removal of the mercaptan by dialysis did not lead to any reaggregation of the smaller protein units as has been noted in the case of sulfhydryl-produced fragments of macroglobulins (11). Treatment of the native \( \gamma \)-globulin with the mercaptan alone effected no discernible size change. Our results on the hydrolysis of normal \( \gamma \)-globulin by papain and the chemical and biological properties (22) of the fragments produced are in keeping with somewhat similar conditions of digestion were used.

Proteins Isolated from Pathological Sera—A study of six serum myeloma proteins indicated that they were split by papain much more readily than normal \( \gamma \)-globulins. Ultracentrifugal diagrams showing the course of the digestion of a typical myeloma protein with only 0.1% of its weight of mercuripapain (with ethylenediaminetetraacetate, \( 1 \times 10^{-4} \) M) are presented as part of Fig. 2. A rapid conversion of molecules of 6.5 S to 3.5 S units is indicated. The amount of nonprotein nitrogen formed in this process is near 1.5% of the protein nitrogen in the system when all of the 6.5 S material has been converted to 3.5 S type molecules. The nonprotein nitrogen liberated does not seem to consist entirely of peptide material and efforts are being made to ascertain its content of various type carbohydrates. Qualitatively, this fraction gives positive tests for hexose, glucosamine, and fucose.

Electrophoretic and ultracentrifuge diagrams of the water-soluble portion of the papain digest of a Waldenström macro- globulin are shown in Fig. 3. Different experiments with the same or different macroglobulins show that variable amounts of material sedimenting between 6.5 and 12 S are produced along with the 3.5 S units. The results presented in Fig. 3 are for a macroglobulin which formed a relatively large amount of water-soluble material sedimenting above 3.5 S.

When sulfhydryl-dissociated macroglobulin components of 6.5 S are digested with papain, all of the protein is converted to 3.5 S material. Treatment with mercaptans of the 3.5 S protein units formed through the action of papain on macroglobulins does not result in a further breakdown to lower molecular weight components.

Chromatography of the various globulins and their papain di-
Fig. 4. The chromatograms of 100 to 150 mg of myeloma protein (I), macroglobulin (II), and γt-pseudoglobulin (III), and their papain digestion products, on carboxymethyl cellulose. All experiments used 2 × 30 cm columns. The constant volume mixing flask contained 125 ml of pH 5.5, 0.05 M sodium acetate buffer; the buffer added was 0.5 M.

gestion products on carboxymethyl-cellulose gave the results shown in Fig. 4. In every papain digestion experiment, an acidic fragment designated A is eliminated with the hold-up volume. Its molecular weight seems to be below 5000 because it dialyzes readily and fails to form a sedimenting boundary at 2.5 × 10^4 times gravity in the ultracentrifuge. The decreased electrophoretic mobility at pH 8.6 of some of the papain digestion products as compared with the parent molecule is understandable in terms of loss of this acidic fragment.

Chromatograms of a myeloma protein and its papain digestion products are shown as part of Fig. 4I. The papain digestion fragments of various myeloma proteins show differences in electrophoretic and chromatographic properties even though they all are cleaved uniformly to molecules sedimenting near 3.5 S and the low molecular weight A fraction. Component B of the myeloma chromatogram at times crystallizes readily when solutions of it are brought to pH 5.5 to 6.0 and dialyzed against distilled water in the cold. A photomicrograph of such material is shown in Fig. 5. Detailed physical studies of Component C showed that it had a sedimentation constant of 3.35 S at zero protein concentration and D_20,w = 9.04 × 10^{-7} cm² sec⁻¹. With a partial specific volume of 0.74, a molecular weight near 34,000 is calculated.

The presence of several electrophoretic components in the papain digests of the myeloma globulins probably indicates that the 3.5 S units vary either in their amino acid composition or their carbohydrate content or both. The amount of carbohydrate in myeloma proteins seems to be related to the isoelectric point (25, 26). The same considerations would apply to the γt-globulin and macroglobulin digestion products which show different charge properties.

Carbohydrate analyses of two multiple myeloma protein systems, by the anthrone method (27), revealed that the lower isoelectric point B fraction of the papain digest of those serum proteins contained from 10 to 20 times more carbohydrate than the more basic protein (Component C). The results are shown in Table I. The RH system is the same as that referred to in Figs. 2 and 4. The myeloma protein, RA, gave papain digest products similar to those obtained with the RH protein. The A fraction of the chromatogram shown in Fig. 4I contains about 3% of the anthrone-reacting material of the parent globulin.

Immunoochemical studies of the papain digest Fractions B and C of the above systems or of similar fractions from other globulins indicate that these molecules are antigenically distinct (22, 23, 28).

It was of interest to determine whether the rate of formation of Component A could be correlated with the rate of conversion of the native 6.5 S protein to 3.5 S material. A 1% solution of the RH myeloma protein was digested at 37° with 0.1% of its weight of papain at pH 5 in the presence of ethylenediaminetetraacetate
(1 × 10⁻² M). One-milliliter samples of the digest were removed at various times and passed through a 0.6- × 8-cm carboxymethyl-cellulose column previously equilibrated with pH 5.5, 0.05 M sodium acetate buffer. The digest sample was washed through the column with the same buffer until 10 ml of effluent had been collected. Under these conditions the acidic A fraction comes through in the hold-up volume but the 3.5 S fractions are held up on the column. The latter was completely eluted by passing 1.0 M NaCl over the column and collecting an additional 10 ml of effluent. The amounts of the acidic A fraction and 3.5 S material were determined in terms of the absorbancies of the two eluates at 280 mμ. The relative amounts of the A fraction released with time of digestion are shown in Fig. 6 and can be seen to be correlated with the rate of appearance of the 3.5 S component shown in the sedimentation experiment reported in Fig. 2. The major portion of the acidic fragment is released in 6 to 8 hours of papain digestion and, concurrently, most of the 6.5 S material is converted to 3.5 S units.

Depicting concentrations of chromatographic components in terms of absorption at 280 mμ is misleading. The acidic fragments formed in the early stages of the papain digestion process, which are eliminated with the hold-up volumes in the chromatograms shown in Fig. 4, give an ultraviolet absorption of the type shown in Fig. 7. The materials studied are the acidic (A) and a mixture of the B and C fractions obtained in the digestion time experiment shown in Fig. 6. The absorption characteristics of the B and C fractions can be seen to show the typical protein absorption maximum near 280 mμ.

The electrophoretic analysis of the water-soluble portions of the papain-digested macroglobulin shown in Fig. 3 likewise indicate that a series of differently charged protein molecules is formed. The chromatographic separation of this material is shown in Fig. 4(11 and is in agreement with the electrophoretic result. Eluting immediately after the acidic Component A were two minor components labeled B and C. These three fractions were of molecular weight of less than 5000, whereas the remaining material sedimented as 3.5 or 10.8 S components or mixtures of these. The low solubility of the native macroglobulin in pH 5.5, 0.01 M acetic buffer is evidenced in Fig. 4(11 by the small peak eluted after the application of 25 ml of a saturated solution of the native protein to the column. The chromatographic separation of a papain-digested γ-globulin that was also reduced with mercaptoethanol after digestion is shown in Fig. 4(111. An acidic A fraction is again seen and several other components are also resolved. The native protein shows a single chromatographic component.

Properties of Fraction A—When lyophilized Fraction A material from myeloma protein (Fig. 4(1) was taken up in a small volume of 10% acetic acid and passed over a column of Sephadex G-25 which had been equilibrated with the acetic acid solution, the main 280 mμ-absorbing peak emerged just before and overlapped the sodium acetate peak; the latter could be detected by the rise in pH of the eluent. Thus, Fraction A consists of material that diffuses into Sephadex nearly as rapidly as the inorganic salt.

The Fraction A material was further examined by rechromatographing it on DEAE-cellulose in pH 5.5 acetate buffer. The result (Fig. 8) shows the material to be heterogeneous. The complexity of the mixture was further established by chromatography on a 0.9- × 40-cm column of Dowex 50-X2, which showed the presence of at least fifteen peptides. Hsiao and Putnam (24) have also noted the release of peptides during the cleavage of γ-globulins by papain.

Amino Acid Compositions of Products from Myeloma Protein—The results of amino acid analyses of the parent protein and Fractions A, B, and C are given in Table II. The A fraction contains only 2.2% of the total residues, and the fraction is, for example, richer in phenylalanine and lower in lysine than the native protein. Fractions B and C account for 28 and 65% of the starting product. The two fractions differ noticeably in amino acid composition; the B fraction contains relatively smaller amounts of alanine and glycine, in particular, and a larger proportion of methionine.

Studies on the sulfhydryl groups of the materials have failed to show the formation of any carboxymethyllysines when the
Fig. 6. The rates of release of a low molecular weight acidic fraction and the formation of 3.5 S units during the papain digestion of a myeloma serum globulin.

Fig. 7. The ultraviolet absorption spectra of the low molecular weight A fraction and 3.5 S protein fragments of a papain-digested myeloma protein.

Fig. 8. Chromatographic separation on DEAE-cellulose of the A fraction from a papain digest of 150 mg of RH myeloma protein. A 2 x 30-cm column was used. The constant volume mixing flask contained 125 ml of pH 5.5, 0.01 M sodium acetate buffer; the buffer added was 0.5 M.

parent protein or the cleaved products are treated with iodoacetate in the presence of denaturing agents. Thus, no evidence for any cysteine residues has been obtained by this method which has given good results for the cysteine residues in hemoglobin (20). On the other hand, spectrophotometric determination with p-chloromercuribenzoate indicated the presence of 7 to 8 —SH groups per 160,000 g with native protein. The same titration gave only 2.5 to 3.5 —SH groups in the total papain digest, and no —SH groups in the products isolated chromatographically. The difference between the results obtained with iodoacetate and p-chloromercuribenzoate remains unexplained.

The possibility that some of the cysteine might be in the form of an acid-labile thiazole derivative was examined by heating the protein with 2 N HCl at 80° for 10 minutes and for 1 hour and applying the iodoacetate reaction in each instance. The brief period of acid hydrolysis did not liberate any cysteine residues capable of reacting with iodoacetate in 8 M urea at pH 9.

Reduction of the protein with sodium borohydride followed by carboxymethylation (29) gave a value of 32 residues of carboxymethylcysteine, a result in fair agreement with the value of 35 residues obtained as cysteic acid after oxidation.

Table II

<table>
<thead>
<tr>
<th>Amino acid†</th>
<th>Residues per protein molecule of molecular weight 160,000*</th>
<th>Total recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Native protein</td>
<td>Found in Component A</td>
</tr>
<tr>
<td>Lysine</td>
<td>91</td>
<td>0.91 (0.9)</td>
</tr>
<tr>
<td>Histidine</td>
<td>28</td>
<td>0.49 (1.8)</td>
</tr>
<tr>
<td>NH₃</td>
<td>132</td>
<td>4.0 (3.0)</td>
</tr>
<tr>
<td>Arginine</td>
<td>36</td>
<td>0.38 (1.1)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>113</td>
<td>2.8 (2.5)</td>
</tr>
<tr>
<td>Threonine</td>
<td>115</td>
<td>2.0 (1.7)</td>
</tr>
<tr>
<td>Serine</td>
<td>166</td>
<td>2.3 (1.4)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>126</td>
<td>3.4 (2.7)</td>
</tr>
<tr>
<td>Proline</td>
<td>112</td>
<td>3.1 (2.8)</td>
</tr>
<tr>
<td>Glycine</td>
<td>98</td>
<td>2.3 (2.3)</td>
</tr>
<tr>
<td>Alanine</td>
<td>80</td>
<td>1.6 (2.0)</td>
</tr>
<tr>
<td>Cystine‡</td>
<td>35</td>
<td>0.9 (2.6)</td>
</tr>
<tr>
<td>Valine</td>
<td>143</td>
<td>3.8 (2.7)</td>
</tr>
<tr>
<td>Methionine</td>
<td>13</td>
<td>0.3 (2.3)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>23</td>
<td>0.5 (2.1)</td>
</tr>
<tr>
<td>Leucine</td>
<td>98</td>
<td>2.5 (2.6)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>67</td>
<td>1.0 (1.5)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>37</td>
<td>1.2 (2.9)</td>
</tr>
</tbody>
</table>

* Numbers in parenthesis are per cent of the residues in the native protein.
† Tryptophan not determined. Qualitatively, the B fraction was lower in this amino acid than the A component. Glucosamine was also present in all of the hydrolysates and was detected chromatographically.
‡ As cysteic acid (17). The value for cystine in the acid hydrolysate of the C component was unusually low, as a result of decomposition during hydrolysis. This seems to be related to a higher level of tryptophan in the C fraction.
a buffered silica gel column. Only the dinitrophenyl derivative of glutamic acid was found in the ether-soluble fraction and the e-lysine derivative in the aqueous-soluble portion. An average of nearly 2 N-terminal glutamic acid residues per 100,000 g of protein was found in both the parent material and in an equivalent amount of the B plus C fractions. No new N-terminal groups were found in the latter fraction. Investigation of N-terminal amino acids by a hydantoin method (32) also revealed no differences between native protein and the B plus C papain digest fractions. Hsiao and Putnam (24) have found new N-terminal amino acids appearing in the 3.5 S fragments produced by the action of sulfhydryl-activated papain on normal human γ-globulin. This subject requires further study.

Analysis for COOH-terminal groups, on the other hand, indicated differences (Table III). The B and C components liberated more amino acids in the given time. The results can be interpreted either as reflecting the presence of new COOH-terminal end groups or a greater availability of the chains originally present.

Previous studies on the COOH-terminal residues of human γ-globulin by hydrazinolysis (33) have indicated serine and glycine to be predominant residues. Traces of alanine, threonine, and glutamic acid were found. Carboxypeptidase released serine, glycine, leucine, and valine with smaller amounts of aspartic acid, glutamic acid, threonine, alanine, and lysine. Comparison of these results with those in Table III indicates similarities between the myeloma protein studied in this work and human γ-globulin Fraction II.

### DISCUSSION

Certain serum globulins found in elevated amounts in pathological conditions are cleaved by papain to units of nearly 35,000 molecular weight more readily than normal γ-globulins. This has also been noted by Hsiao and Putnam (24) for some myeloma globulins. A cleavage to similar size molecules by combinations of (a) urea and reducing agents (34), (b) pepsin and reducing agents (21), and (c) reducing agents and mild acid conditions (35) suggests the possibility that bonds other than or in addition to peptide are cleaved by papain. One would normally expect papain, as a proteolytic enzyme, to effect a degradative reaction by means of hydrolysis of peptide bonds. Several features of the experiments on the myeloma protein support the view that such is the case in this instance. The low molecular weight fraction, consisting as it does of small peptides, may contain new NH2-terminal residues that apparently are liberated by proteolytic action. The 3.5 S units, in turn, seem to have new COOH-terminal residues. This result may indicate that four 3.5 S units may be attached through their COOH-terminal sections to some central connecting core in the macromolecule. The analytical results for N-terminal amino acids in a variety of myeloma proteins do not support this view in the majority of cases. From one to five N-terminal groups per mole have been reported (38). This is not consistent with a molecule made up of four subunits.

Although mercuripapain catalyzes the cleavage of globulins without any deliberate activation of the enzyme by reducing agents, the addition of Versene (the disodium salt of ethylenediaminetetraacetic acid) does increase the rate of splitting. The use of cysteine-activated papain, however, markedly increases the rate. Confirmatory results have been reported by Hsiao and Putnam (24).

### TABLE III

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Myeloma protein</th>
<th>B and C components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>0.06</td>
<td>2.64</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.60</td>
<td>1.54</td>
</tr>
<tr>
<td>Aspartic</td>
<td>0.40</td>
<td>0.94</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.54</td>
<td>0.90</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.17</td>
<td>1.92</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>Aspartic</td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>

*4-hours incubation at 37°C.*

Tests made in the course of the present studies have shown that, under the conditions used to digest serum globulins, mercuripapain had from 0.5 to 1.0% of the activity of fully activated papain towards benzoylarginine amide. This indicates that limited proteolysis of the myeloma proteins could take place. However, the data of Fig. 6 show that, when the conversion of myeloma protein to 3.5 S units is complete, no further formation of the peptide fraction takes place. This is not indicative of a small amount of protease acting on a large amount of substrate.

The possibility that sulfhydryl groups in the globulins could activate the papain was investigated. The slight cleavage of benzoylarginine amide by mercuripapain in the absence of reducing agent was not augmented by the presence of the myeloma protein. Kimmel and Smith (37) have reported that mercuripapain is inactive for synthetic substrates and that Versene alone did not cause an activation.

Mercuripapain is known to cleave thioesters near pH 5 without special activation (38) and one might consider whether hydrolysis of a thioester, or perhaps a thioacetal or thioglycoside, might be taking place. Such a reaction remains a possibility but the attempts to demonstrate such linkages of cysteine were negative.

Studies of the 3.5 S fragments obtained, both with papain and nonproteolytic methods (34, 35), would be essential before we could conclude that papain and reducing agents might be capable of splitting the same bonds in the myeloma proteins. The 3.5 S units produced by the action of papain on macroglobulins undergo no further degradation on treatment with mercapto-ans. This may indicate that in this case the bonds cleaved by papain are identical with those split by reduction and acidification (pH 4 to 4.5) of the macroglobulin.

### SUMMARY

Normal γ-globulins, myeloma, and macroglobulinemic serum proteins may be degraded by papain to molecules of molecular weight near 35,000. In each instance there is also split off a low molecular weight peptide fraction in small yield (2 to 3%).

The three chromatographically separated fractions of a papain digest of myeloma protein have been subjected to more detailed study and compared with the parent molecule. The two 35,000 molecular weight fractions show differences in carbohydrate and amino acid composition. When the two products are compared with the original myeloma globulin, there is evidence for new or more readily available carboxyl-terminal groups, but no evidence...
for new amino-terminal residues. The liberation of a peptide fraction which contains at least fifteen peptides suggests that these account for new amino-terminal groups.

Acknowledgments—The experiments on the amino acid compositions and chemical properties of Fractions A, B, and C from the myeloma protein were carried out during the time that one of us (H. F. D.) was visiting the Rockefeller Institute as a George Haight Traveling Fellow from the University of Wisconsin. He is indebted to Dr. Stanford Moore and Dr. William H. Stein for advice and the most hospitable donation of the facilities of their laboratory during this phase of the study.

REFERENCES

Action of Papain on Human Serum Globulins
H. F. Deutsch, E. R. Stiehm and Jane I. Morton


Access the most updated version of this article at http://www.jbc.org/content/236/8/2216.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/236/8/2216.citation.full.html#ref-list-1